

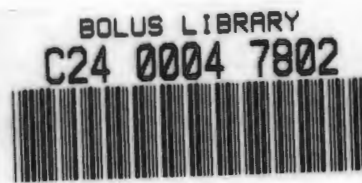
**DEFINING THE SPECIES BOUNDARIES WITHIN  
THE *R. BOLUSII* COMPLEX  
USING MORPHOLOGICAL CHARACTERS**

**Terry Morley**

**Botany Honours  
Systematics Project  
Supervisor: Prof. H.P. Linder  
University of Cape Town  
1994**

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.



## DEFINING THE SPECIES BOUNDARIES WITHIN THE *RESTIO BOLUSII* COMPLEX USING MORPHOLOGICAL CHARACTERS

### ABSTRACT

A group of species occurring within the genus *Restio* that are commonly referred to as the *Restio bolusii* complex were examined to see if their boundaries could be defined on the basis of macro-morphological characters. Six species have been assigned to this complex. They are *R. bifurcus* Nees ex Masters, *R. bolusii* Pillans, *R. burchellii* Pillans, *R. insignis* Pillans, *R. praeacutus* Masters and *R. strobilifer* Kunth. Specimens were obtained from the Bolus Herbarium of the University of Cape Town. Sixty-one morphological characters were examined in twenty-six specimens.

Analysis involved the use of three techniques: cluster analysis using the UPGMA method, ordination using Principal Component Analysis and cladistic analysis. These revealed three groups to consistently cluster together which corresponded to the species *R. bolusii*, *R. strobilifer* and *R. praeacutus*. *R. insignis* formed a sister group to the *R. strobilifer* group in the phenograms and tended to group within the *R. strobilifer* group in the ordinations. Cladistic analysis also indicated it to be part of this group. Clearly definable characteristics could not be found to circumscribe the remaining two species.

However the clear delimitation of the three groups mentioned above was thwarted by the occurrence of a group of specimens that showed a combination of characteristics that caused them to be intermediate between the more clearly defined groups. These specimens clustered weakly and inconsistently in the phenograms and were seen to occupy a central position in the ordinations.

## INTRODUCTION

The Restionaceae consist of rush-like, tufted or rhizomatous, evergreen plants with photosynthetic culms and leaves reduced to sheaths (Dahlgren and Clifford, 1982; Linder, 1984). The family is wind pollinated and mostly dioecious, often with morphological differences occurring between male and female. Distribution of the family is largely in the southern hemisphere with the majority of species occurring in the southwestern parts of South Africa and Australia.

The genus *Restio* includes 80 species and constitutes the largest genus within the Restionaceae, but the group has not as yet been shown to be monophyletic and genera such as *Platycaulos* and *Calopsis* may have to be included (Linder, 1984).

The *Restio bolusii* complex includes *R. bifurcus* Nees ex Masters, *R. bolusii* Pillans, *R. burchellii* Pillans, *R. insignis* Pillans, *R. praeacutus* Masters and *R. strobilifer* Kunth. This group is characterised by: bracts that are closely imbricate and which are rounded to acute but never acuminate at the apex; a band of dark hollow cells that occur distally on the bracts; lateral tepals of the outer perianth whorl that are villous on the carina and conduplicate. An historical background to the species descriptions is cited in table 1. These are largely based on macromorphological characters with the exception of Cutler (1969) which describes culm anatomy and Linder (1984) which also includes micro-morphological characters, phytochemistry and culm anatomy.

Species of this complex are found in the winter rainfall region of the South Western Cape on nutrient poor soils. The distribution of *R. bifurcus* has been described as being in low lying sandy areas on the Cape Peninsula reaching north to Malmesbury and south to Caledon (Linder, 1985). *R. bolusii* has a more montane distribution from Caledon to Worcester and Stellenbosch. *R. burchellii* is reported to occur in mountains from Villiersdorp to Hermanus over a large altitudinal and moisture range. *R. insignis* specimens are from the Cedarberg and *R. praeacutus* specimens are from low lying sandy areas mainly in the Malmesbury division. *R. strobilifer* is a high mountain species found from the Cedarberg to the Hex River mountains.

**TABLE 1: Previous descriptions of species within the *R. bolusii* complex**

<u>Species</u>	<u>Author</u>	<u>Year</u>
<i>R. strobilifer</i>	Kunth; p.398	1841
	Masters; p.282	1878
	Pillans; p.248	1928
	Cutler; p.271	1969
	Linder	1984
	Linder; p.461	1985
<i>R. bifurcus</i>	Masters; p.247	1865
	Masters; p.275	1878
	Masters; p.83	1897
	Pillans; p.245	1928
	Pillans; p.133	1950
	Cutler; p.260	1969
	Linder	1984
	Linder; p.442	1985
<i>R. praeacutus</i>	Masters; p.84	1897
	Pillans; p.246	1928
	Cutler; p.268	1969
	Linder	1984
	Linder; p.457	1985
<i>R. bolusii</i>	Pillans; p.247	1928
	Cutler; p.260	1969
	Linder	1984
<i>R. burchellii</i>	Linder; p.442	1985
	Pillans; p.340	1942
	Linder	1984
<i>R. insignis</i>	Linder; p.442	1985
	Pillans; p.251	1945
	Linder	1984
	Linder; p.450	1985

The species boundaries of this group have not been adequately resolved nor is it certain what ranking be assigned to the taxa as little in depth morphological or other analysis has been performed on them.

The criteria used to delimit a species is controversial in systematics. Concepts based on pattern involve the unique combination of characters and those on process involve the potential for gene exchange and natural selection. Character based species concepts tend to be the more widely accepted ones at present such as the phylogenetic species concept in which species are delimited on the basis of unique and constant character combinations (De Queiroz and Donoghue, 1988; Nixon and Wheeler, 1990; Davis and Manos, 1991). The characters that are useful in the delimitation of species are those that have become genetically fixed as opposed to characters that vary as a result of interbreeding and recombination. This study is based on the phylogenetic species concept to search for fixed differences in morphology.

The question addressed in this study is whether the distinction of these species can be justified on macro-morphological grounds. Quantitative analysis of specimens obtained from the Bolus Herbarium of the University of Cape Town was performed in an effort to seek unique characters by which groups within the complex can be identified. Phenetic analysis was first performed to investigate the occurrence of distinct clusters produced in the phenetic space which may be due to phylogenetic divergences and thus can be used to identify separate species (Crisp and Weston, 1993). However, other factors may also result in distinct clustering such as sex differentiation and ontogenetic differences and this needs to be prevented by the separate analysis of males and females in the Restionaceae and by examining specimens at the same stage of development. Cladistic analysis was also performed on certain of the specimens.

## **MATERIALS AND METHODS**

### **Materials**

Specimens were obtained from the Bolus herbarium of the University of Cape Town. All herbarium sheets with specimens denoted as belonging to this complex were initially grouped on the basis of similar distributions, flowering times and similar superficial resemblances. More groupings were obtained than there are species names for this complex as groups were formed which appeared to differ only in distribution or flowering time. As too many herbarium sheets were available for detailed analysis, one to four from each grouping were selected for detailed analysis. Specimens were chosen that were at a similar stage in development, namely when the stigmas and stamens were fully developed but prior to seed development. Table 2 lists the specimens examined and indicates their altitude, flowering time and distribution. For cladistic analysis, only those specimens that were well separated, as indicated by the phenetic analysis, were used.

### **Measurements of macro-morphological characters**

Measurements were taken with Vernier callipers with an accuracy to 0.1 mm. Spikelets were softened by boiling in soapy water, dissected and examined under a dissecting microscope.

The Operational Taxonomic Units (OTUs) in the study refer to individual herbarium sheets. The number of measurements per OTU depended on the maximum number that could be taken per sheet and so varied between sheets.

The initial set of characters measured are shown in appendix I. For quantitative data minimum and maximum values were recorded in addition to the average values to provide an idea of the range within and the overlap between each OTU. Distribution, flowering time and altitude were also noted in addition to these characters but were not included as part of the data set.

TABLE 2: Details of specimens (= OTUs) used in the study

Specimen Number	Herbarium Sheet Reference no.	Initial Grouping	Altitude (m)	Flowering Time†	Distribution
1	R. strobilifer Hutchinson 592a	I	600	2	Gorge West of Ceres
2	R. strobilifer Levyns 4695	I	600	2	Ceres
3	R. strobilifer EE‡ 8968	I	1500	2	Waaiohoek Peak Worcester
4	R. strobilifer EE 13065	II	1400	2	Southern Cedarberg
5	R. strobilifer EE 11303	III	1700	2	Wemmershoek Peak Paarl
6	R. strobilifer Levyns 11709	IV	800	2	Above Dasklip Pass
7	R. strobilifer EE 13968	V	1000	2	Swartberg Ladismith
8	R. insignis Stokoe 8470	VI	1400	2	Cedarberg
9	R. insignis Levyns 985	VI	1500	2	Matroosberg De Doorns
10	R. bolusii Stokoe 17664	VII	?	1	Wilde Paarde Berg Worcester
11	R. bolusii EE 34543	VII	900	1	Galgeberg Robertson-Caledon
12	R. bolusii Stokoe 7176	VII	1400	1	Mountain above Genadendal
13	R. bolusii EE 35110	VIII	1000	2	Blokkop Villiersdorp
14	R. bolusii EE 35698	VIII	1000	2	Franschhoek Pass
15	R. bifurcus Parker 4876/7	IX	200	2	Inland from Pringle Bay
16	R. bifurcus Stokoe 1340	IX	100	2	Between Palmiet River & Kleinmond
17	R. bifurcus EE 31564	IX	100	2	Between Brackenfell & Kraaifontein
18	R. burchellii EE 35537	X	500	2	Vogelgat Reserve Hermanus
19	R. burchellii EE 31037	X	600	2	Nuweberg Forest Reserve, Caledon
20	R. burchellii EE 33658	X	900	2	Jonkershoek Forest Reserve
21	R. strobilifer Taylor 10304	XI	800	1	Between Nuweberg and Boesmanskloof
22	R. praeacutus EE 34750	XII	low-lying	2	Near Mamre Malmesbury
23	R. praeacutus Bolus 12896	XII	30	2	Hopefield
24	R. praeacutus EE 35265	XII	low-lying	2	Pella Reserve Malmesbury
25	R. praeacutus Linder 5186	XII	100	2	Hill between Silverstroom and Ganzekraal
26	R. praeacutus EE 11296	XIII	1500	2	Wemmershoek Peak Paarl

† 1 = early flowering specimens; 2 = late flowering specimens

‡ EE = E. Esterhuysen



For sheath lengths, measurements were taken to both the distal edge of the coriaceous section and to the tip of the mucro. This was because, although the former measurement was preferable due to the mucro often being worn away or completely lost, in certain specimens it was not clear where the coriaceous section ended and the mucro began. In the latter instance the length to the tip of the mucro was the only one that could be taken objectively.

Two measurements of spikelet length were also included (fig. 1). The first measurement was taken from the base of the spathe as there is a very obvious line from which to measure and was termed the 'total length'. The other measurement was taken from more or less the base of the first bract rows where the spikelet starts to swell outwards and termed the 'spikelet length'. This was to account for the fact that the spathe may be situated well below the bulk of the spikelet on some specimens. On other specimens the two measurements were very similar and one can barely discern between them.

Deciding on the base of the spikelet in the latter measurement involved an element of subjectivity thus all subsequent measurements with regard to spikelet size were taken with respect to the spathe base. Unfortunately, this had the effect of causing the basal width measurements (Character nos. 46 - 48) to sometimes be misleading. For example, in figure 2 the basal width measurement would be less than the apical one giving the false impression that the spikelet was widest towards the apex. To account for this an additional character was included that stated the position of the widest diameter (Character 60).

The term decay cells was used to refer to a band of dark hollow cells that occur at the apex of the bracts, sometimes spreading onto the coriaceous section. This feature is found only in this complex and *R. filiformis*. Other terminology used in conjunction with the bracts is indicated in figure 3.

Many of the characters in this data set are measures of the same phenomenon and could be seen as redundant (Abbot *et.al*, 1985). This would have the effect of weighting these repeated characters in the analysis. The data set was thus amended to remove repetitious



FIG. 1: Length measurements of the spikelet.

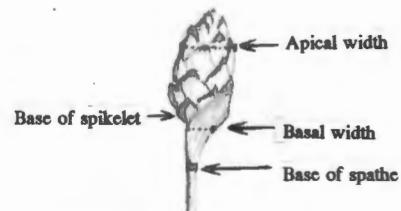


FIG. 2: Measurements taken a quarter of the way from the apex and from the base of the spikelet to provide an indication of shape

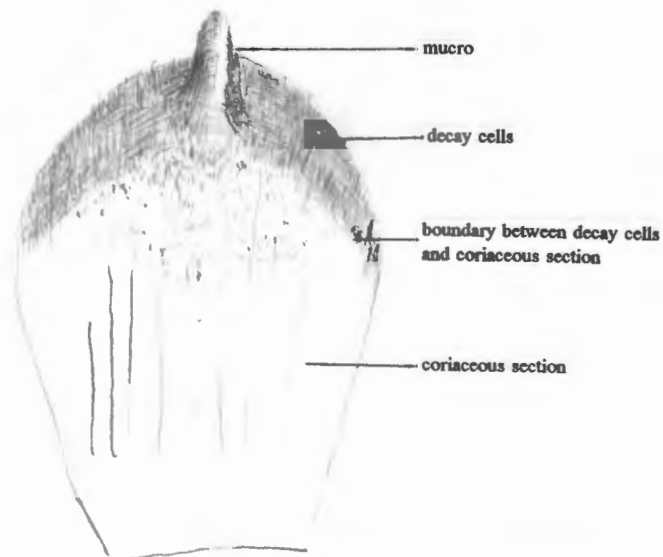


FIG. 3: Terms used in connection with the bracts

characters (appendix II). Spikelet and bract measurements were changed to ratios of the total spikelet and bract lengths respectively thus giving new characters related to shape rather than size. Average values only were retained and range values excluded.

The character states related to the sheath mucro (character 7 of appendix II) are illustrated in figure 4. Examples of the variation in bract features are shown in figure 5. The female flower typical of the group is illustrated in figures 6 and 7.

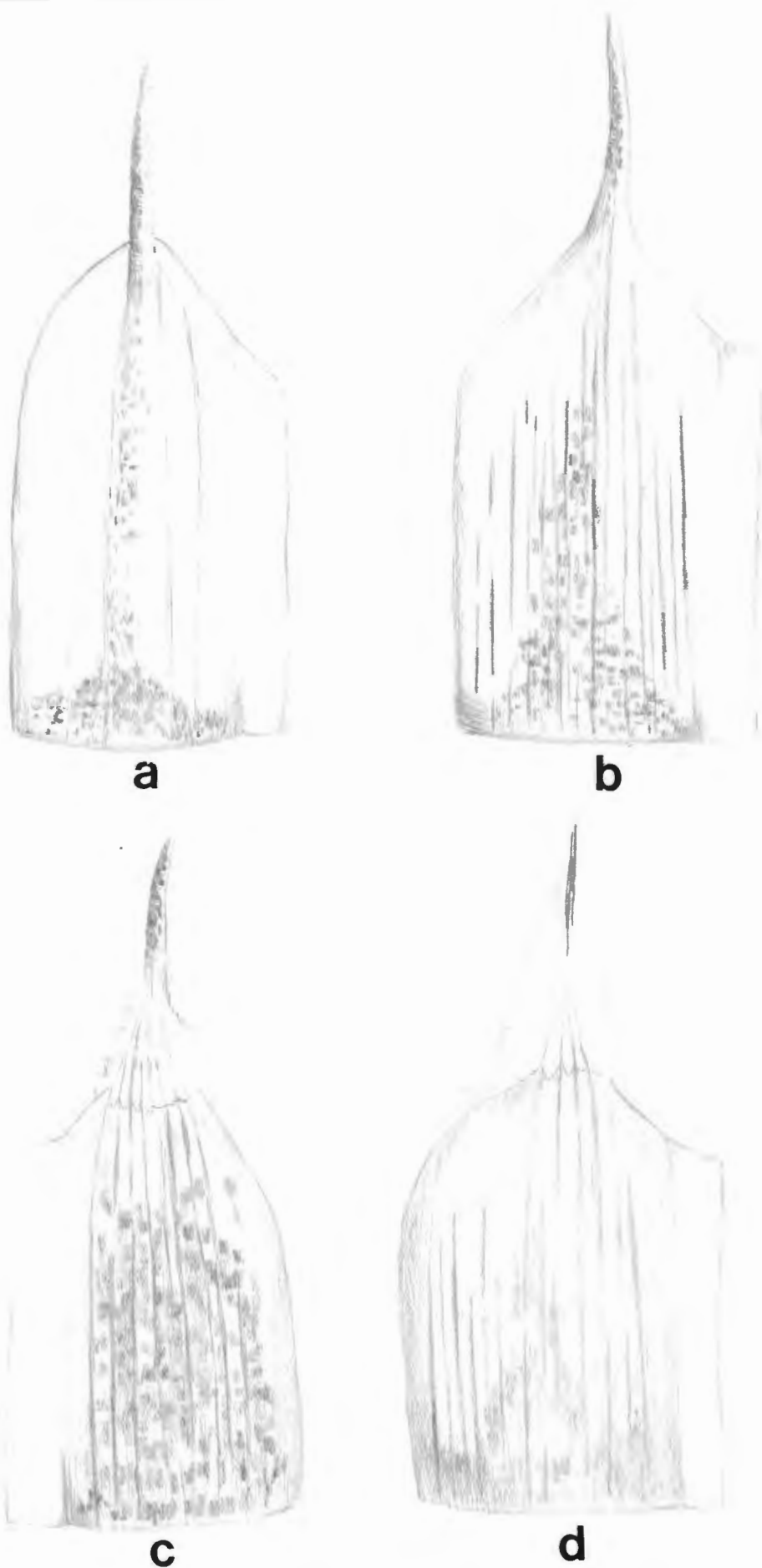
### Anatomical characters

A selection of previously prepared anatomical cross-sections of culms were examined under the compound microscope to determine variation in culm anatomy. These sections had been cut using a sledge microtome and stained with safranin-alcian blue. The slides examined are available from the Botany Department of the University of Cape Town and are catalogued as 16.64 *R. strobilifer* Esterhuysen 34846; 16.74 *R. bifurcus* Parker 4750; 17.05 *R. praeacutus* Stokoe 7338; 17.15 *R. bolusii* 31522a; 17.31 *R. insignis* Stokoe 9562 and 17.43 *R. burchellii* Esterhuysen 28998.

### Analysis of macromorphological characters

The techniques employed in quantitative analysis depend on what pattern of relationship expected within the study group (Crisp and Weston, 1993). Populational differences can be reticulate and clinal but not necessarily hierarchical whereas species differences are expected to be hierarchical (Nixon and Wheeler, 1990; Davis and Nixon, 1992). If differences are attributable to population differences, the results from cladistic and phenetic cluster analysis, which produce hierarchical models, may display high levels of homoplasy and will lead to erroneous conclusions (Abbot *et.al*, 1985).

Crisp and Weston (1993) found ordination to be most suitable for indicating variation not due to speciation as it can reveal multiple, continuous and overlapping patterns of variation. Cluster analysis is unable to reveal secondary variation patterns, for example that due to ontogenetic differences, that may be confusing the primary pattern, whereas ordination may reveal such patterns. In this study the annual nature of the reproductive



**FIG. 4:** The various types of mucro found extending from the sheath. a. cylindrical mucro arises abruptly from sheath; b. cylindrical mucro that tapers onto the sheath; c. cylindrical mucro with membranous base; d. flat membranous mucro held together by veins.

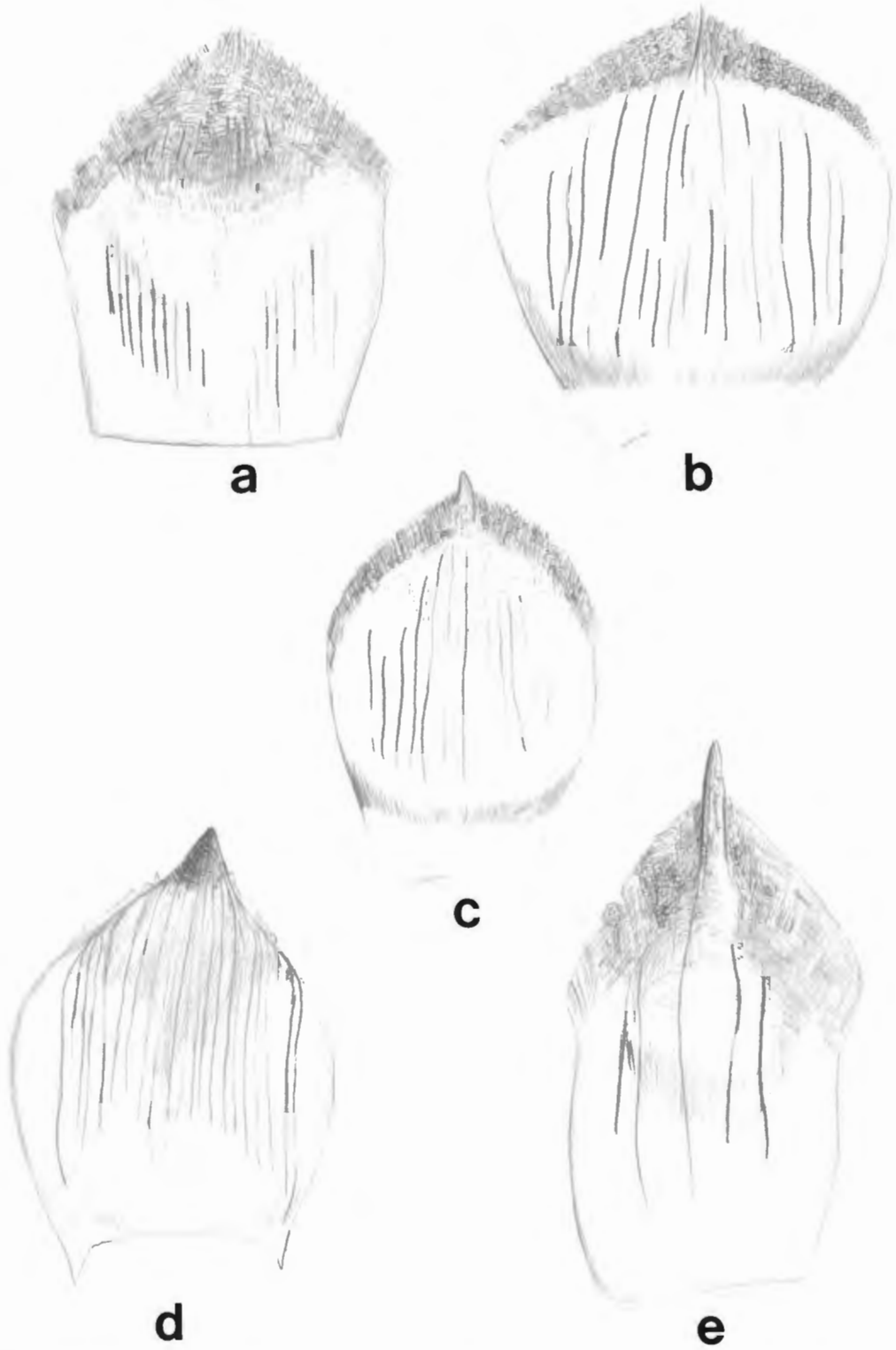


FIG. 5: Variation in bracts found within the *R. bolusii* complex. a. muticous; b. slight ridge in area of decay cells; c. mucro forms hook in area of decay cells; d. broad mucro with base on coriaceous section; e. slender mucro with base on coriaceous section.



**FIG. 6: The female flower**



**FIG. 7: Parts of the female flower.** The first row of drawings are of the outer perianth segments with the anterior tepal placed centrally and flanked by the lateral tepals. The second row is of the inner perianth whorl with the posterior tepal in the centre. The bottom drawing is of the three styled ovary. Note the reduced stamens.

culms and the use of species all at anthesis should prevent the occurrence of ontogenetic differences.

There is often uncertainty, however, whether differences can be attributed to population differences or speciation. In the *Restio bolusii* complex a great variety of characters can be observed leading one to expect species differentiation but there is uncertainty as to whether all six species could be thus differentiated, thus a variety of techniques including ordination, cluster analysis and cladistics were employed.

### Phenetic Analysis

Phenetic variation patterns among the specimens were analyzed using the software package NTSYS-pc (Rolf, 1993). Numerical phenetics can be used to define groups on the basis of the greatest number of shared characteristics. Such an evaluation does not include any interpretation of shared similarities in an evolutionary context. It employs multivariate statistical methods and is suitable for metric and ordered multistate data (Abbot *et.al*, 1985). Both qualitative and quantitative data are included in the analysis but as the qualitative data is ordered it may be transformed into quantitative data (Abbot *et.al.*, 1985).

Metric quantitative data includes all measurements related to lengths and widths. Discontinuous, quantitative data includes the number of spikelets per culm, number of flowers per spikelet, number of bract rows and number of veins in the bracts and various tepals. These are all counts and thus have ordinal and interval properties.

Qualitative data is used to describe the sheath and bract mucros, shapes of spikelet and bract apices, the position of the widest diameter of the spikelet, the boundary between the decay cells and the rest of bract, persistence of decay cells and branching pattern. The data are generally ordered, linear and can be handled like quantitative data (Abbot *et.al*, 1985) although problems were encountered in describing apices. The states identified were rounded, elliptic, obtusely and acutely angled. However, the state could progress from rounded to elliptic just as easily as from rounded to obtusely angled and from either obtusely angled or elliptic to acutely angled but did not generally appear to



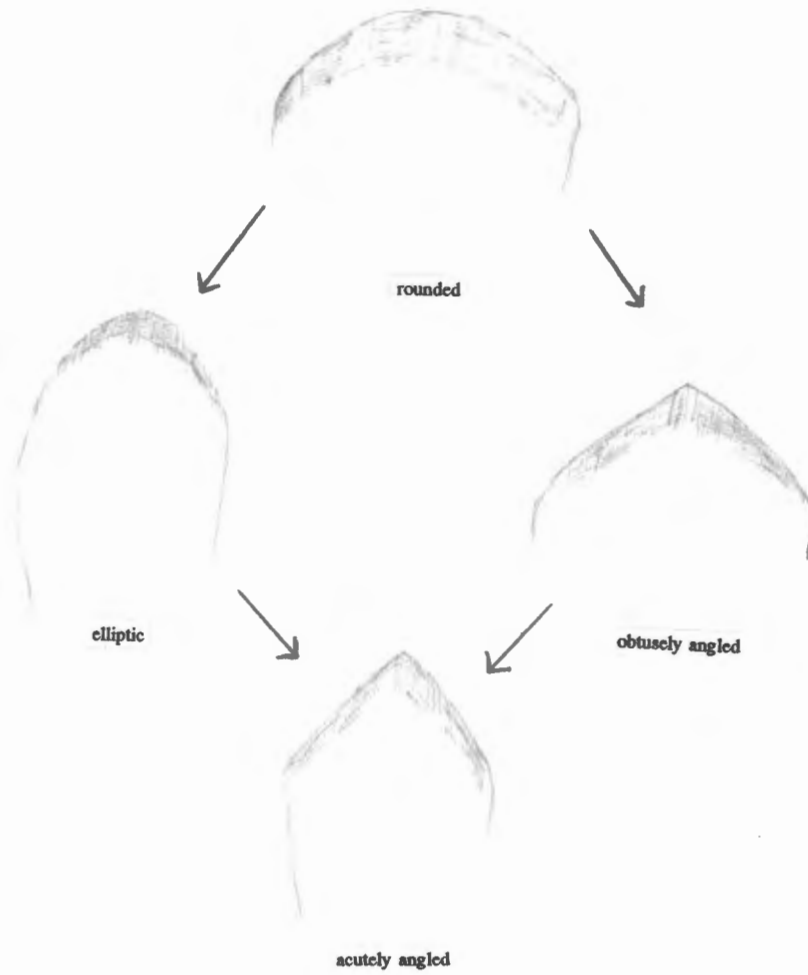
progress from obtusely angled to elliptic (figure 8). As this character is not strictly ordered and may cause problems in the analysis, the program was rerun with these characters delimited in alternative ways as well as being excluded from the analysis to see the effect this would have on the outcome.

Analysis was also performed with characters related to the male bracts excluded as this resulted in a data set with very little missing information.

For the cluster analysis, NTSYS-pc (Rolf 1993) was used to standardize the data matrix to enable the comparison of diverse characters, calculate a dissimilarity matrix using the Manhattan distance measure, and generate a phenogram with the unweighted pair-group method using arithmetic averages (UPGMA). This method utilises the arithmetic means of all items in a cluster to determine the distance between it and a new candidate for the cluster rather than simply using the closest single linkage (Abbot *et.al*, 1985). This method of clustering is most frequently used as it produces the best phenograms as measured by the cophenetic correlation coefficient, a measurement often used as a means of evaluating the effectiveness of clustering (Sneath and Sokal 1973; Duncan and Baum, 1981). The objective of cluster analysis is to hierarchically group items according to a distance or similarity measure (James and McCulloch, 1990).

The ordination method used was Principal Components Analysis (PCA) from which two dimensional models of the morphological variation was constructed. A correlation matrix of characters was constructed to calculate eigenvectors for the components.

The objective of ordination is to provide a graphical description of a data set of items and their attributes (variables) by reducing its dimensions and graphically displaying correlations of variables that illustrate the maximum variation between the items (James and McCulloch, 1990). Values of variables are assumed to be linear and that there are no interactions. Most of the variation is usually summarised with only a few components, so data with many variables can effectively be displayed on a two or three dimensional graph.



**FIG. 8: Bract apices**

Loading values were used to determine which characters were contributing most to the variation. Loading refers to the correlation of an original variable with one of the linear combinations constructed by the analysis. Thus high negative and positive loadings are useful in determining which characters are contributing to the variations displayed by the ordination.

### **Cladistic Analysis**

Cladistic analysis, which uses parsimony to construct a hierarchical arrangement of the OTUs (Davis and Nixon, 1992), was performed using 32 of the characters (Appendix III). The objective of such an analysis is the discovery of nested sets of monophyletic groups that is presumed to be the outcome of evolution. The computer program *Hennig 86* written by J.S. Farris was used to locate the set of shortest trees and *Clados* written by Kevin Nixon was used to for the graphical representation.

The sensitivity of the tree to changes in the data set was tested by the bootstrap and Permutation Tail Probability (PTP) tests. The bootstrap method (Felsenstein, 1985; Sanderson, 1989) tests the sensitivities of individual nodes. In order for it to be effective, characters used in the analysis must be independent. Inclusion of related characters causes an overestimation of confidence as cladograms formed from such a data set will be much less likely to correspond to random data sets even if cladistic covariation does not occur.

The PTP is a test of cladistic covariance in a data set and is defined as "the estimate of the proportion of times that a tree can be found as short or shorter than the original tree" (Faith and Cranston; 1991). The null hypothesis is that the observed most parsimonious tree could have been produced from chance alone. Significant cladistic covariation exists if the PTP is less than a prescribed value which is commonly taken as 0.05.

If the null hypothesis is not rejected this does not mean that the observed data are the result of random processes but rather that the existence of a cladistic structure cannot be confirmed. Rejection of the null hypothesis however can lead to the conclusion that

cladistic covariation does occur. In this study random reshuffling of the character states among the OTUs was repeated to produce 100 new data sets each of which were used to calculate a most parsimonious tree for the PTP test.

## **RESULTS**

### **Initial Grouping**

The initial grouping of the herbarium specimens resulted in the formation of thirteen groups as described below.

I. Single to two spikelets per female culm and one to four per male culm; male spikelets with rounded to elliptic bases and pointed apices; female spikelets more elliptic basally and with a more sharply pointed apex; muticous bracts with obtusely angled apices and dark band of decay cells; very closely imbricate bracts, especially those of the females. Specimens occurring at high altitude in the vicinity of Ceres and Worcester.

II. Very similar in superficial appearance to group I but tending to possess fewer and larger male spikelets and with a more northerly distribution in the Cedarberg.

III. Male spikelets more elliptic than group I; mucronate bracts with less well formed area of decay cells. Superficial resemblance to group VIII.

IV. Much smaller male spikelets than the above three groups with more per culm; muticous bracts. Specimens from high mountain areas of the Clanwilliam and Worcester divisions.

V. More easterly distribution with specimens occurring in the Swartberg. Superficially most similar to group IV. Female culms with more spikelets than in the above groups; band of decay cells less obvious.

VI. Culms less branched, often being single; spikelets ovate with very rounded base and pointed apex; much fatter spikelets than group I and single to gemini in both male and female; muticous bracts. Specimens mainly from the Cedarberg but also further south in the Hex mountains.

VII. Bracts with rounded apices and short stout mucros; coriaceous section with a more striated appearance. Autumn flowering whereas all other groups except group XI flowered in Spring. Collected from high mountain areas from Caledon to Worcester.

VIII. Similar in appearance to group VII but flowering in Spring. Bracts with a less striated appearance. Similar distributions to group VII.

IX. Plants tending to occur at a lower altitude. The culm was often, but not always, slightly flattened and sulcate. This feature was occasionally noted in most of the groups but occurred less often than in this group. A mucro was present but less well formed than in the eighth group. Specimens were reported from low-lying areas of the Cape Peninsula and Stellenbosch and North to Malmesbury. However, there were also some higher altitude specimens in the Caledon area.

X. Similar in appearance to group IX but occurring at higher altitude. Bracts generally more rounded apically. Distribution in the Worcester and Caledon areas.

XI. Similar in appearance to group VII but with mucro less well developed. Specimens were recorded from the Caledon area.

XII. Less tightly imbricate bracts than the above groups and spikelets generally smaller. Occurring at low altitude in the Malmesbury division.

XIII. Similar to group XII but found at higher altitude with specimens all collected from the Paarl division, mainly Wemmershoek peak.

### **Phenetic Analysis**

Cluster analysis of the data compiled from the complete set of characters described in appendix I produced the phenogram shown in figure 9.

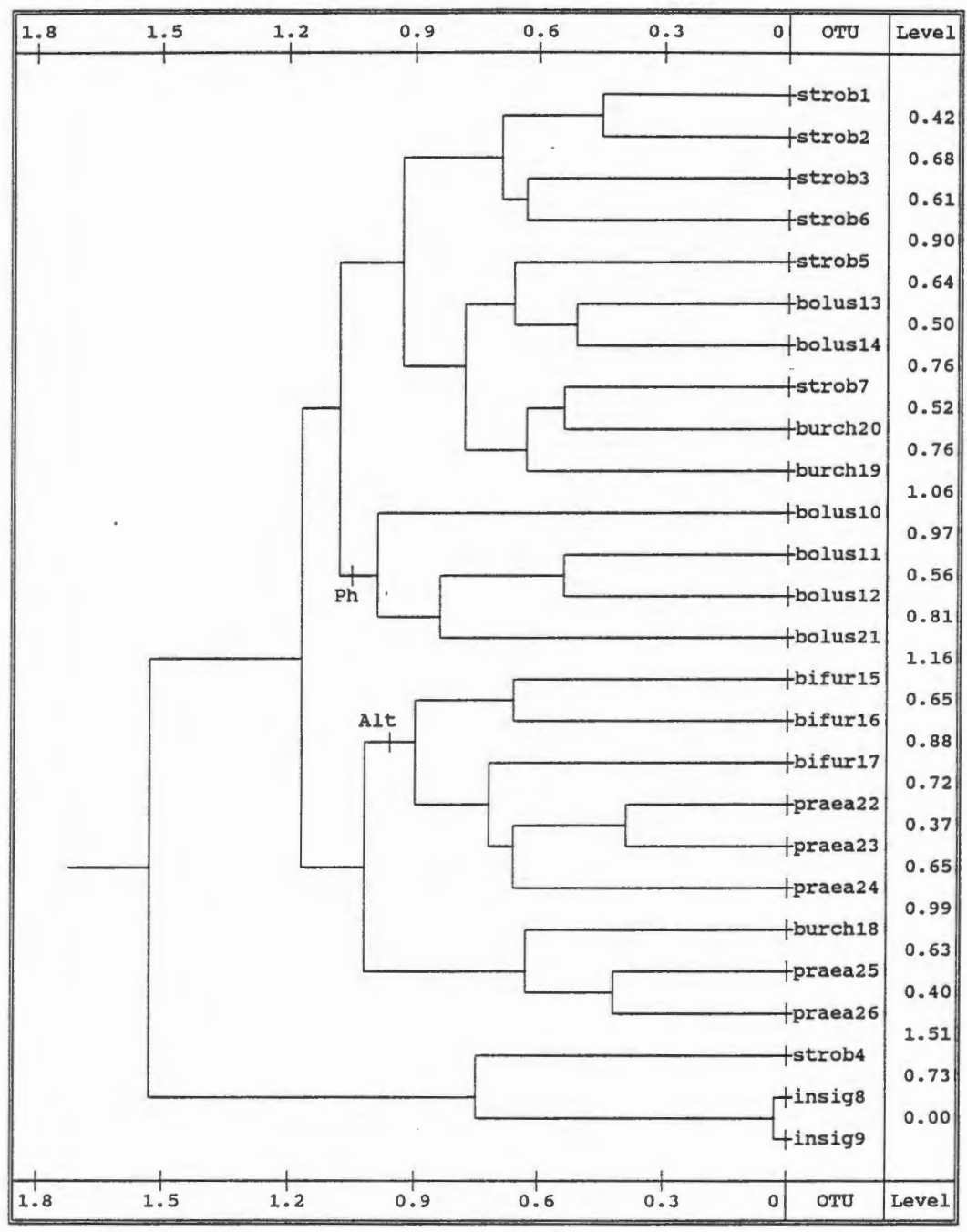


FIG. 9: UPGMA phenogram using Manhattan distance based on the complete set of characters for specimens within the *Restio bolusii* complex. Clusters were formed which correspond to altitude and phenology.  
Alt = altitude (low altitude group); Ph = phenology (Autumn flowering group).

Principal component analysis of these characters is illustrated in figure 10. The variables contributing most to the separation in the first and second components are indicated in table 3 in order of highest loadings.

The data set compiled from the characters selected to reduce measurements of the same phenomenon (Appendix II) is presented in Appendix IV. This data set produced the cluster diagram in figure 11. The ordination produced by principal component analysis is illustrated in figure 12. Characters contributing most to the separation as indicated by highest loadings are listed in table 4.

Alternative ways of delimiting character states associated with the bract apices or removal of these characters due to their not being strictly ordered did not have a significant effect on the clustering or ordination patterns.

Analysis with male characters excluded caused better groupings in certain instances as evidenced by lower linkage levels in the phenogram (fig. 13) and closer groupings on the ordination (fig.14).

### **Anatomy**

The only anatomical differences noted included the lack of peg cells in the first row of chlorenchyma cells in *R. bifurcus* and protective cells that only extended to the junction between the two rows of chlorenchyma cells as opposed to reaching to the parenchyma sheath in the *R. praeacutus* specimen. However, Cutler (1969) reported that the protective cells do extend all the way to the parenchyma sheath in the latter species as well as being found to occur extending only to the junction of the two chlorenchyma layers. Otherwise, the culm anatomies were found to be very conservative and so no anatomical analysis was included.



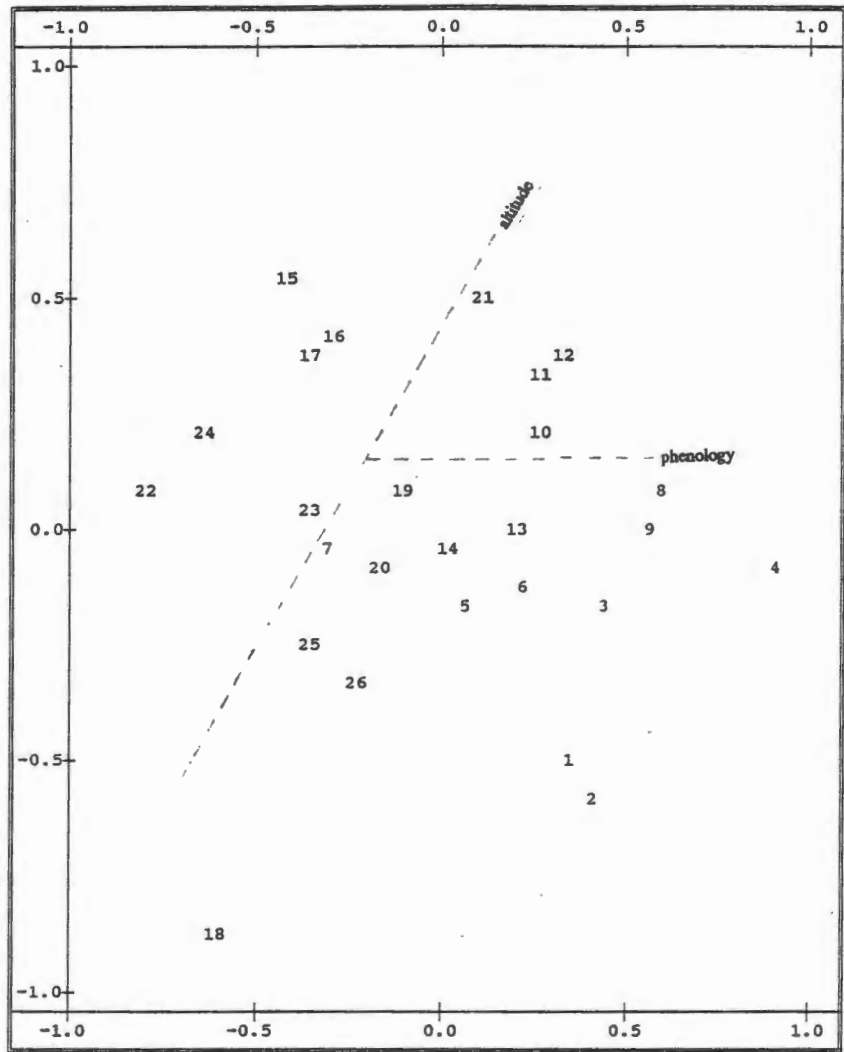


FIG. 10: Two dimensional principal components analysis based on the 118 characters of the complete set of characters (Appendix I) for 26 OTUs. Dashed lines on the ordination indicate the separation of OTUs according to altitude and phenology.

**TABLE 3: Variables contributing most to separation of specimens within the ordination depicted in figure 10. The variables listed are those with the 15 highest loading values. Number indicate the character number as listed in Appendix I.**

<b>FIRST COMPONENT</b>	<b>Loading value</b>
55. Average spathe length including spike (F)	0.919
90. Maximum halfway width of spikelet (M)	0.893
88. Average halfway width of spikelet (M)	0.885
114. Number of veins per bract (M)	0.870
37. Average total length of spikelets (F)	0.863
40. Average spikelet length from base of bracts (F)	0.858
56. Minimum spathe length including spike (F)	0.854
39. Maximum total length of spikelets (F)	0.852
38. Minimum total length of spikelets (F)	0.846
42. Maximum length of spikelets from base of bracts (F)	0.829
97. Average spathe length (M)	0.827
47. Minimum basal quarter width (F)	0.825
94. Average apical quarter width (M)	0.823
41. Minimum spikelet length (F)	0.822
95. Minimum apical quarter width (M)	0.810
<b>SECOND COMPONENT</b>	
20. Maximum penultimate sheath length including mucro (F)	0.945
17. Maximum penultimate sheath length excluding mucro (F)	0.924
18. Maximum ultimate sheath length including mucro (M)	0.921
26. Maximum ultimate sheath length including mucro (M)	0.917
32. Maximum penultimate sheath length including mucro (M)	0.912
12. Average ultimate sheath length including mucro (F)	0.907
23. Maximum ultimate sheath length (M)	0.905
14. Maximum ultimate sheath length including mucro (F)	0.881
25. Minimum ultimate sheath length including mucro (M)	0.864
15. Average penultimate sheath length (F)	0.862
24. Average ultimate sheath length including mucro (M)	0.862
9. Average ultimate sheath length (F)	0.861
29. Maximum penultimate sheath length (M)	0.858
3. Maximum diameter of stem (F)	0.854
21. Average ultimate sheath length (M)	0.846

F = female; M = male

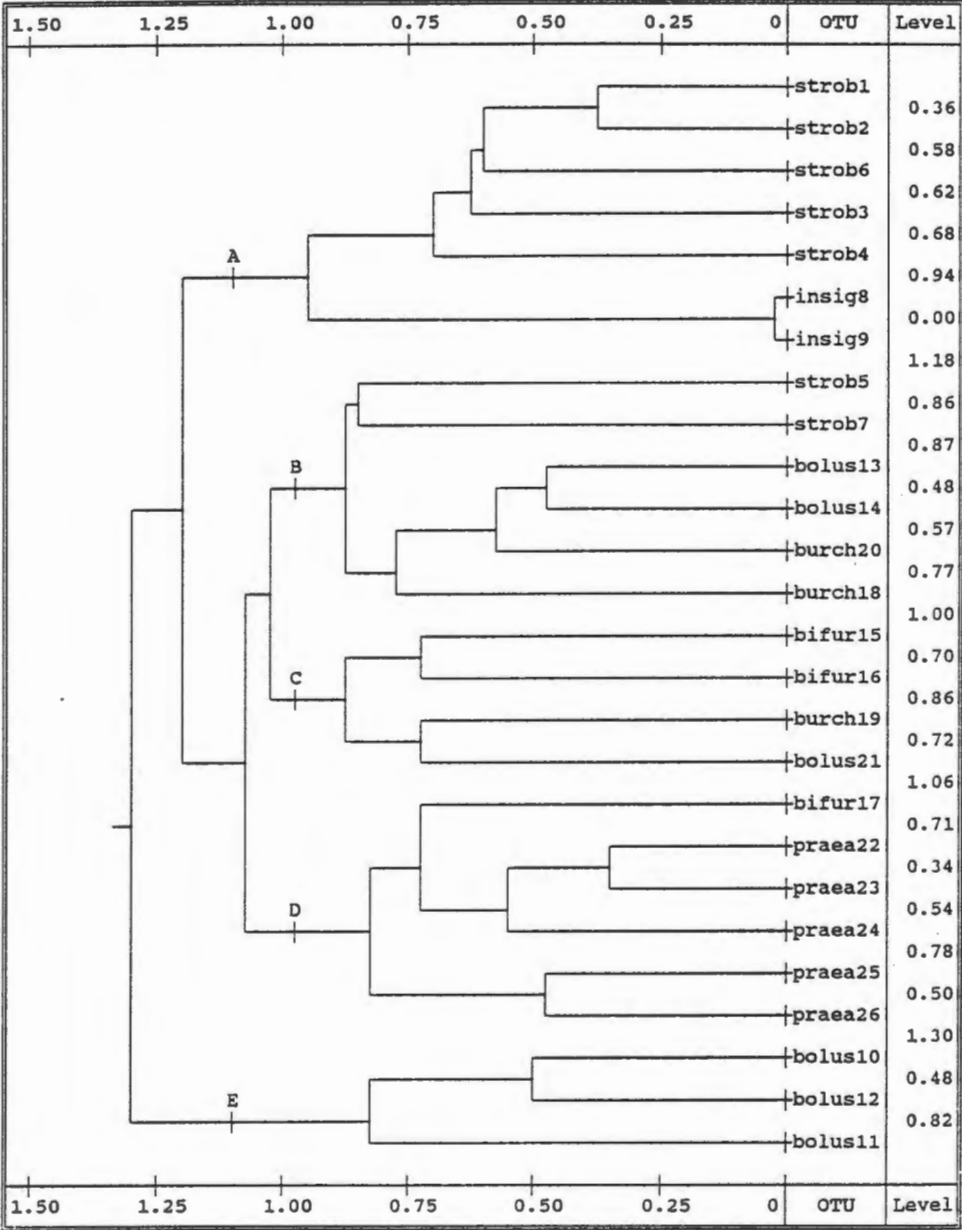


FIG. 11: UPGMA phenogram using Manhattan distance based on the selected set of characters for specimens within the *Restio bolusii* complex.

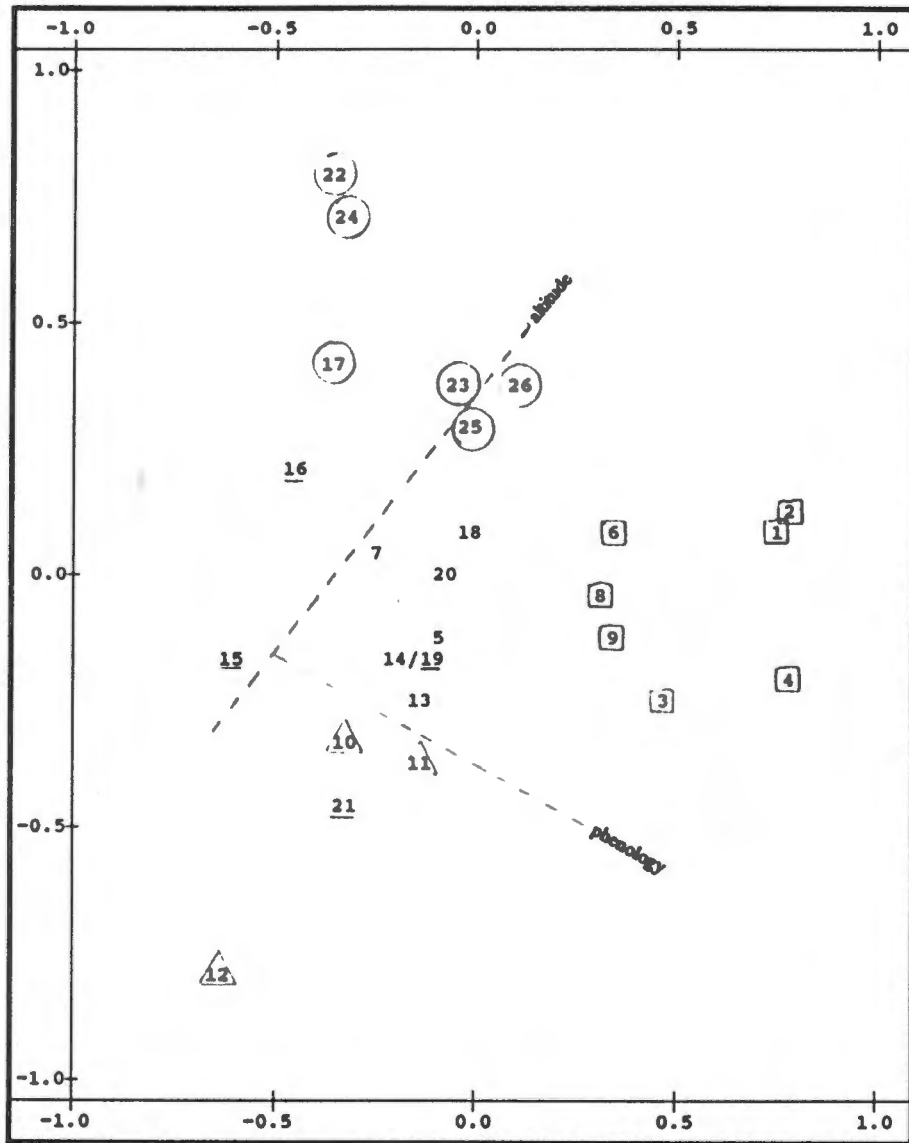


FIG. 12: Two dimensional principal components analysis based on the 61 characters of the selected set of characters (Appendix II) for 26 OTUs. Dashed lines on the ordination indicate the separation of the OTUs according to altitude and phenology. The first component accounts for 23.73% of the variance and the second component for 16.69%. (The z axis accounts for a further 11.45%).

□ = Group A; Unmarked = Group B; — = Group C; ○ = Group D; △ = Group E.

**TABLE 4: Variables contributing most to the separation of specimens in the ordination depicted in figure 12. The first fifteen variables are listed in order of highest loading values. Numbers indicate character number according to Appendix II.**

<b>FIRST COMPONENT:</b>	<b>Loading values</b>
46. Number of bract rows per spikelet (M)	0.873
45. Position of widest diameter on spikelet (M)	-0.864
18. Number of bract rows per spikelet (F)	0.830
52. Bract mucro (M)	-0.816
30. Number of flowers per spikelet (F)	0.756
44. Base of spikelet (M)	-0.741
58. Number of flowers per spikelet (M)	0.735
53. Ratio of mucro length to bract length (M)	-0.726
61. Venation of anterior tepal (M)	-0.720
24. Bract mucro (F)	-0.713
37. Total length of spikelet (M)	0.709
9. Total length of spikelet (F)	0.706
16. Base of spikelet (F)	-0.695
17. Position of widest diameter of spikelet (F)	-0.678
57. Number of veins per bract (M)	0.658
<b>SECOND COMPONENT</b>	
23. Bract apex at distal end of coriaceous section (F)	0.869
29. Number of veins per bract (F)	-0.814
27. Boundary between decay cells and coriaceous section (F)	-0.724
57. Number of veins per bract (M)	-0.630
41. Ratio of spikelet apical width to total length (M)	-0.629
35. Venation of posterior tepal (F)	-0.618
51. Bract apex at distal edge of coriaceous section (M)	0.608
34. Venation of lateral tepals of interior whorl (F)	-0.592
33. Anterior tepal venation (F)	-0.590
50. Bract apex at distal edge of decay cells (M)	0.586
22. Bract apex at distal edge of decay cells (F)	0.584
55. Boundary between decay cells and coriaceous section (M)	-0.584
21. Ratio of widest part of bract to length (F)	-0.563
8. Number of spikelets per culm (F)	0.545
28. Persistence of decay cells (F)	0.533

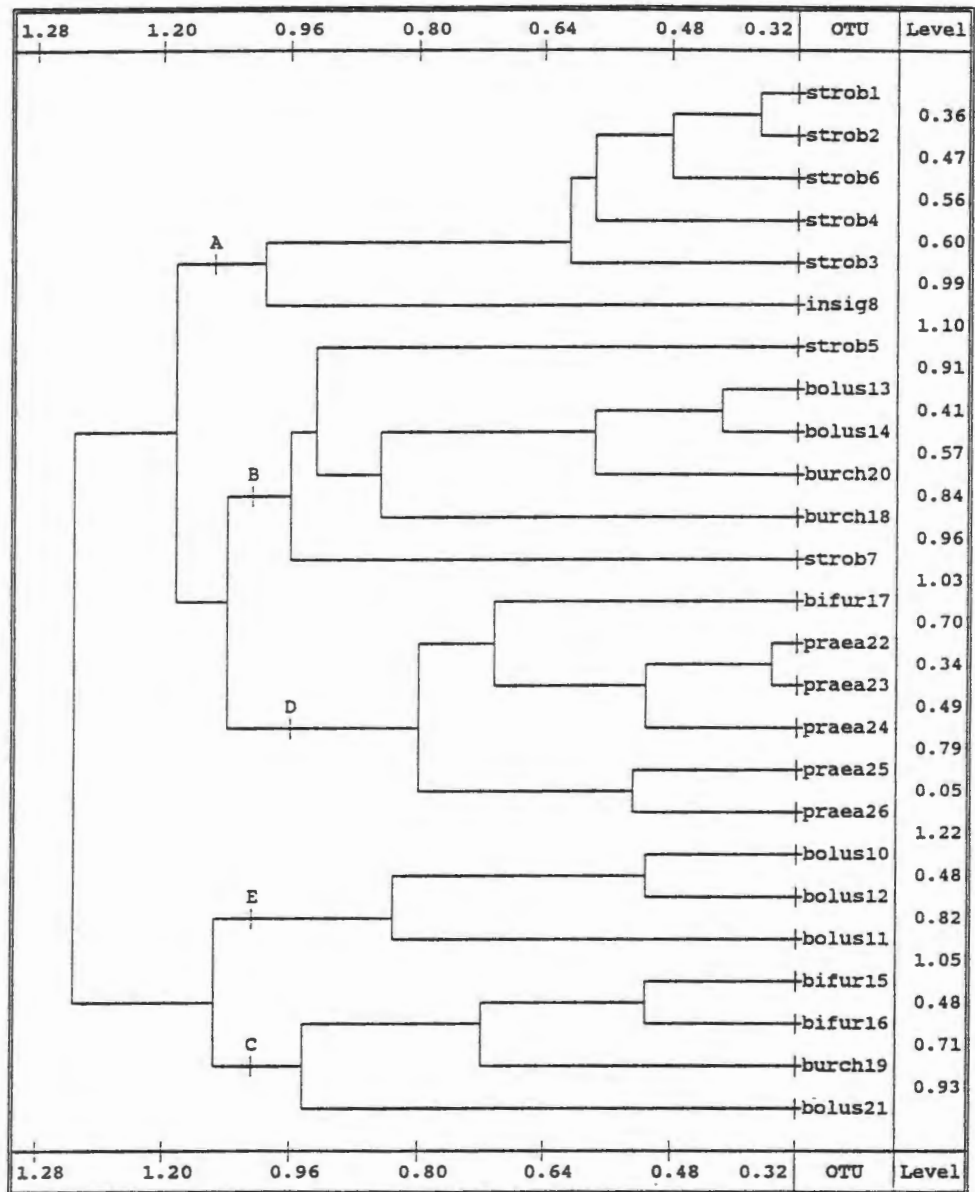


FIG. 13: UPGMA cluster analysis using Manhattan distance based on the 32 characters related to the female plants only.

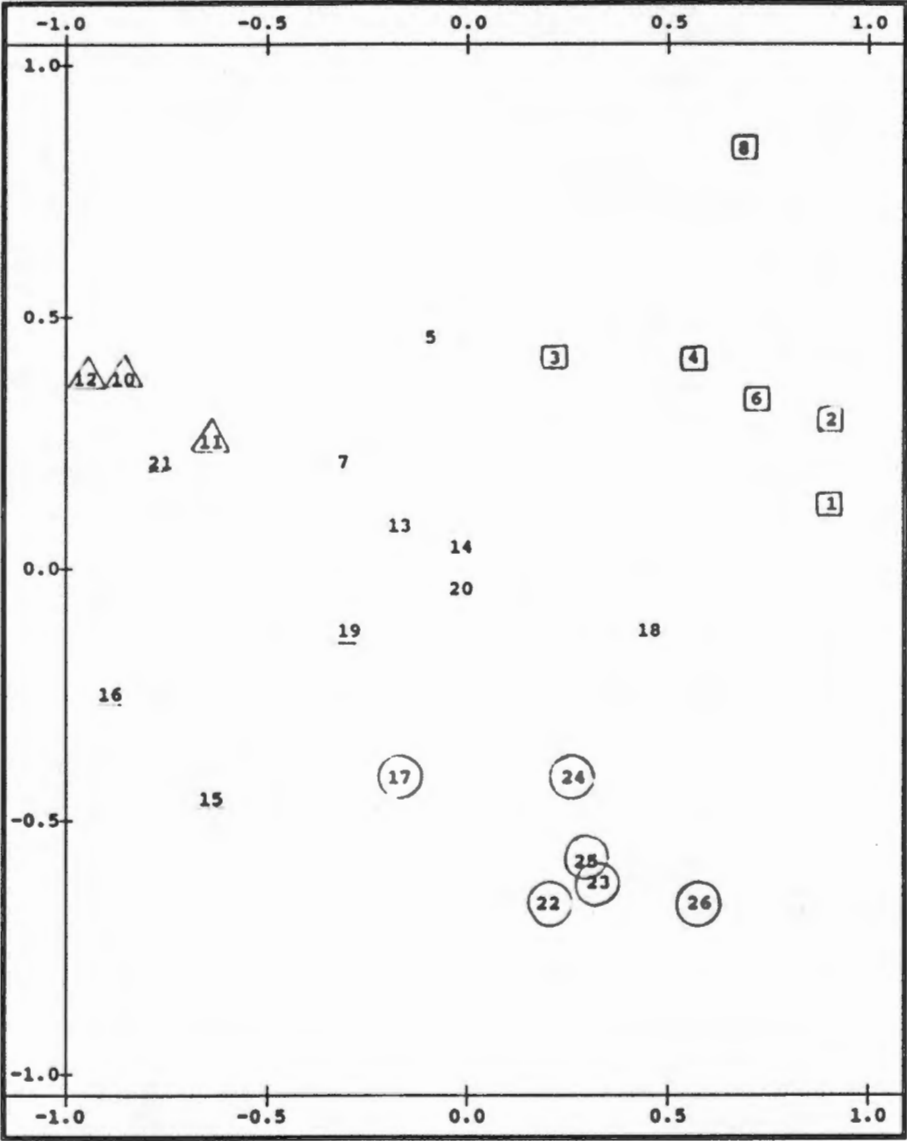


FIG 14: Two dimensional principal components analysis based on 32 characters related to the female plants.  
□ = Group A; Unmarked = Group B;    = Group C; ○ = Group D; △ = Group E.

### Cladistic Analysis

Cladistic analysis was applied to only the outerlying groups as indicated in the ordinations, that is, all items with the exclusion of strob5, strob7, bolus13, bolus14, burch18, burch19 and burch20. A specimen of *R. filiformis* was used as the outgroup. Nine equally parsimonious cladograms were produced which had a length of 145 steps. A strict consensus cladogram is illustrated in figure 15. The consistency index was 48 and the retention index was 71.

The support for each of the nodes as indicated by the bootstrap method is illustrated in figure 16. The range of tree lengths obtained for random data sets produced by the PTP test ranged from 229 to 212 steps. The probability of such a data set randomly producing a tree length of 145 steps was effectively zero ( $p = 0.009901$ ). Thus it is highly unlikely that such a tree can be formed by chance alone so cladistic covariation appears to be occurring.





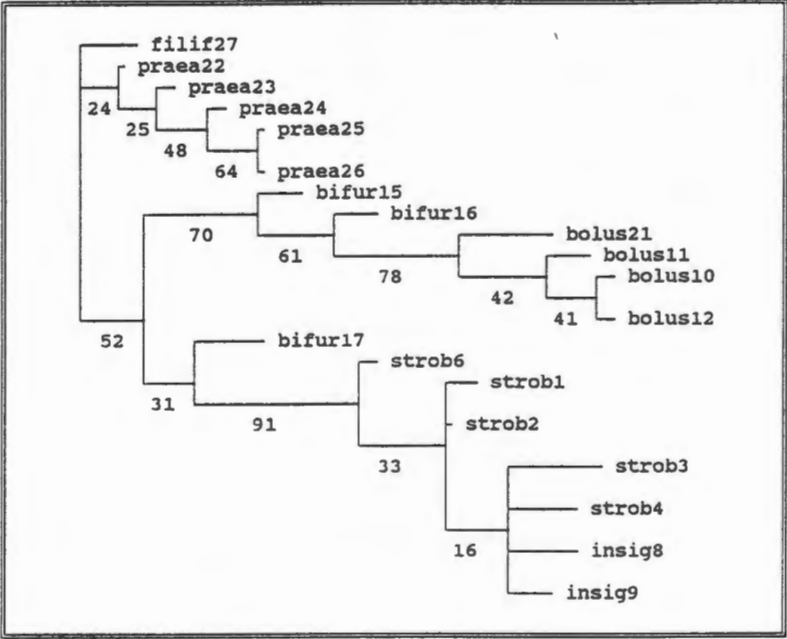


FIG 16: Support for each of the nodes of the strict consensus cladogram of fig. 15 as indicated by the bootstrap test.

## **DISCUSSION**

### **Phenetic Analysis of Complete character set**

In the phenogram (fig. 9) a group is formed that includes all the early flowering specimens and another group that contains all those at low altitude with the exception of praea25. This latter specimen is, however, described as growing on a hill, albeit a low one at only 100 m, whereas all the other *R. praeacutus* specimens are described as occurring in low-lying sandy areas. The ordination (fig. 10) also indicates that placement of specimens can be delimited in terms of altitude and phenology. Thus morphological characters support the separation of the specimens in terms of altitude and phenology as these factors were not included as characters in the data set.

This cluster diagram and ordination is, however, probably highly influenced by environmental factors. The loading values in table 3 indicate that many of these characters are measurements of the same phenomenon. This is especially true of the second component which includes characters that are nearly all related to sheath size. Thus the OTUs are being grouped largely on the basis of size measurements which will obscure any underlying pattern of grouping due to independent attributes. For example, examination of the characters indicates that strob4, insig8 and insig9 are separated from the other specimens as a result of a number of characters indicating the possession of larger female spikelets.

In this phenogram and all subsequent ones the two *R. insignis* specimens are seen to cluster very closely together. This is the result of very little overlap in comparable attributes as insig8 consists of female culms and insig9 of male culms only.

### **Phenetic Analysis of Selected Character Set**

Phenetic analyses based on characters selected to prevent character dependence, indicates the occurrence of five groupings. The nodes of these groups have been labelled as A

through to E in the phenograms (figs. 11 and 13) and will be referred to as such to facilitate discussion.

Group A appears to correspond to a *R. strobilifer* group with the *R. insignis* specimens forming a sister group. Group B is an ill-defined group that contains a variety of morphological features. Group C contains two of the *R. bifurcus* specimens as well as burch19 and bolus21. The other analyses, ordination and cladistics, as discussed below, tended to place this group differently as will be discussed later. The specimens of group D, with the exception of praea26, are all found to occur at low altitude. Praea25 consistently grouped closest to the high mountain specimen, praea26. The remaining low altitude specimens, bifur15 and bifur16, did not group with these specimens as in the first phenogram but were included in group C instead.

The early flowering '*bolusii*' consisting of bolus10, bolus11 and bolus12, clustered together as they did in the first phenogram, to form group E. Linkage levels are lower indicating closer grouping. However, the remaining early flowering specimen, bolus21, which clustered with this group in the first phenogram, is now excluded.

Principal component analysis, as with the first ordination, again demonstrates that specimens can be demarcated according to altitude and phenology. The low altitude specimens are not clearly separated from each other in figure 12 but in the analysis of female plants only they appear to form two groups more tightly clustered than previously, with bifur17 forming an intermediate between them.

Group A forms a cluster in the ordination that is positioned towards the higher positive values of the first component. The *R. insignis* specimen is found to separate out from the rest of the group on the second component when only characters of the female plants are included (figure 14).

The specimens of group B are placed centrally within the ordinations and examination of their characters indicate it to form a very mixed group with the individuals showing various characteristics of the other outerlying groups. This group thus prevented the

formation of clear boundaries between the groups or the occurrence of characters that were unique to a group.

Group C is not supported by the ordinations with bolus21 now being placed with the other early flowering specimens and burch19 forming part of the central group. Group E plus bolus21 thus corresponds to an autumn flowering group. Bifur15 and bifur16 are now grouped with the low altitude specimens again.

### **Cladistic Analysis**

Cladistic analysis, which was performed with the items of the centrally placed, mixed group excluded, appears to agree more closely with the ordinations rather than the phenograms with the exception that the major bifurcation occurs between Group D excluding bifur17 (ie. all the *R. praeacutus* specimens) and the remaining specimens (fig. 15). The basal node of the group excluding the *R. praeacutus* items is well supported by a number of unique characters.

Group A is found to form a clade with the separation of *R. strobilifer* from *R. insignis* not supported. Bifur17 is indicated as ancestral to this group. Group E also forms a clade which, as in the ordinations, includes the other autumn flowering specimen (bolus21), with bifur15 and bifur16 appearing to be ancestral to the group.

### **Characteristics of the various groups**

#### **Characters distinguishing high and low altitude specimens**

The specimens that occurred at low altitude possessed more spikelets per culm on the female plants. Items at high altitude mostly bore one to two spikelets per culm with maximum values of 4 to 6 occurring in the centrally positioned, mixed group only (group B plus burch19). The low altitude species had an average of 6.3 to 9.6 spikelets per culm with a range from 3 to 17. Male spikelets also showed this tendency but it was not as obvious, with averages in the high altitude items of 1.2 to 4.8 and in the low

altitude items of 9 to 18.5. However, the range of values at high altitude is greater with values of up to 12 spikelets per culm being noted.

The high altitude species, with the exclusion of the middle group, also tended to have larger spikelets with a total spikelet length of 18.6 mm to 26.1 mm in the females, whereas the low altitude groups were 11 mm to 16.6 mm long. Group B, with all specimens occurring at high altitude, spanned the gap between these two groups with length measurements of 10.7 mm to 21.4 mm. Male spikelets were on average 10.4 mm to 22 mm long in the high altitude specimens (excluding the middle group) and 8.8 mm to 14.2 mm in the low altitude specimens. Thus the degree of overlap is much greater in the male characters.

#### The *R. strobilifer/insignis* group

Group A appears to correspond to *R. strobilifer*. No clear distinction occurred between this group and *R. insignis* in this study as opposed to Linder (1985) in which the separation between the latter species and *R. bolusii* was uncertain. This group had larger spikelets than the other groups with an average total spikelet length in females of 22.1 mm to 26.1 mm in female plants and 13.5 mm to 22 mm in male plants. The other groups showed values of 10.7 mm to 21.4 mm in female plants and 8.8 mm to 15.1 mm in male plants. Spikelets are widest near the base with a sharply acute apex.

The bracts of this group are muticous (fig. 5 a) or possess a slight ridge. A specimen within the middle group prevented the possession of muticous bracts being unique. The bract apex was widely obtuse in female spikelets and rounded to widely obtuse in male spikelets. The female bract length tended to be longer than in the other groups (as also described in Pillans (1928)) with lengths of 8 mm to 10 mm whereas the lengths of bracts in the other groups tended to be shorter but there was considerable overlap in this character preventing it being useful.

The number of flowers possessed by this group is greater than the other groups with 11 to 43 flowers in female plants and 13 to 52 in male plants. The other groups possessed 1 to 11 flowers in the female plants and 9 to 20 in the male plants.

The female anterior tepals all possessed one central vein with none to one occurring in the males. This was the only group to possess no veins in the tepals. The tepals of the inner whorl exhibited one central vein as well with the exception of strob3 which displayed three veins in all these tepals. The female anterior tepal was glabrous with the exception of strob4 which exhibited slight apical villousness. The male anterior tepals are not always glabrous as described in Pillans (1928). Thus although the usual state is glabrous this is not a completely reliable character.

Items within this group that were assigned to *R. insignis* were noted to possess larger female spikelets and more flowers per spikelet in both male and female plants than the items assigned to *R. strobilifer*. The spikelets of the former were fatter with a ratio of width to total length of 0.43 in the female spikelets as opposed to 0.22 to 0.27 in the *R. strobilifer* specimens. The male *R. insignis* spikelets exhibited a ratio of 0.42 as opposed to 0.32 to 0.37 in the rest of the group. The female spikelet contained 43 flowers and the male 52, whereas 11 to 26 female flowers and 13 to 33 male flowers per spikelet occurred in the remaining items of the *R. strobilifer* group. Spikelets were ovate with much more rounded bases in the specimens designated as *R. insignis*. Fewer veins were also noted in the female bracts with only 7 occurring as opposed to 14 to 21. A greater range of sampling would clarify whether there is a continuum between the character states of the *R. strobilifer* and *R. insignis* specimens.

### **The *R. praeacutus* group**

Within the low altitude species a group could be discerned that displayed fairly constant features which will be referred to as the *R. praeacutus* group. This largely corresponds to group D in the phenogram with the exception of bifur17. The typical sheath mucro was that of diagram a in figure 4 although type b was also found to occur. The sheath displayed in diagram a appeared to be unique to the low altitude items.

The bract of *R. praeacutus* is illustrated in figure 5 e. It has a slender mucro, a distal decay cell area that is retained intact, an indistinct boundary between the coriaceous section and the area of the decay cells, and only three to five veins.

The anterior tepal possessed very few (about three), very short, barely noticeable villi that extended from the tip of the tepal with the exception of that within the female spikelet of praea22 that was only pigmented in this region. This was a unique feature as villousness, where it occurred in other groups, consisted of a long entanglement of villi that were situated on the main body of the tepal.

The *R. praeacutus* group also displayed constancy in tepal venation in that all tepals of both males and females always possessed one central vein only whereas the remaining low altitude species were extremely variable showing one to seven veins per tepal.

The high altitude specimen within this group, praea26, together with the hill top specimen, praea25, were noted to possess the unique character of being much more highly branched than the other items. They also have only one or two female spikelets whereas all the other lower lying specimens of *R. praeacutus* had many.

#### ***R. bifurcus* and *R. burchellii***

The remaining low altitude items (bifur15, bifur16 and bifur17) were more variable in terms of the sheath mucro than the *R. praeacutus* group, with both a and d types occurring (fig. 4).

Greater variability also occurred with regard to the female bract shape, which exhibited elliptic to narrowly obtuse apices and a mucro that formed a small hook within the decay cell area (fig.5 c), or a more substantial mucro as in diagram d (fig.5) except that decay cells were persistent. The possession of a distinct boundary to the coriaceous section was not constant. The male bracts were rounded to widely obtuse at the distal edge of the coriaceous section and the boundary was always distinct. More veins tended to occur in the female bracts than those of *R. praeacutus*, with 7 to 12 commonly occurring, whereas male bracts displayed 3 to 9.

Villousness was also more variable in these remaining low altitude species with females being non-villous to densely villous apically.



Pillans (1928) differentiated *R. praeacutus* from *R. bifurcus* by a glabrous anterior tepal and terete culms in the former species and apical villousness and furrowing of the culms on drying in the latter. *R. burchellii* has also been differentiated from *R. bifurcus* as being rarely villous and having a terete culm (Linder, 1985). *R. burchellii* was differentiated from *R. praeacutus* in that in the former the dark hollow cells (decay cells) were sharply delineated from the coriaceous section and possessed a rounded apex whereas the latter species was said to have no clear delineation and an acute apex (Linder, 1985).

In this study, *R. praeacutus* conforms to the former descriptions of this species, but *R. bifurcus* and *R. burchellii* are not clearly resolved. A variable combination of villousness and the nature of the boundary of the decay cell area occurs within the low-lying specimens bifur15, bifur16 and bifur17 and in those specimens that were placed centrally in the ordinations.

Careful examination of the characters of bifur15, bifur16 and bifur17, indicates that bifur15 and bifur16 share a number of features indicating the possibility that they correspond *R. burchellii sensu* Pillans (1942) whereas bifur17 corresponds to *R. bifurcus* (Pillans; 1928). The former two items possess only one flower per female spikelet which at 9 mm is larger than that of the other groups. This flower was also atypical of the complex as a whole in that the lateral tepals of the outer perianth whorl were not conduplicate and could only be distinguished from the anterior tepal by the presence of villousness. Bifur17 was found to possess five flowers of the usual type. Pillans (1942) described *R. burchellii* as only possessing one to three flowers whereas *R. bifurcus* is described as possessing about four flowers (Pillans, 1928). Bifur17 is also very densely villous on the female anterior tepal whereas those of bifur15 and 16 are glabrous thus corresponding to previous descriptions of the two species. However, all male anterior tepals are villous.

Bifur15 and bifur16 also possessed similar sheath and bract mucros (type a in figure 4 and type e in figure 5 respectively) whereas bifur17 has a different sheath and bract mucro (type d in figure 4 and type c in figure 5 respectively). Bifur17 possessed only

one central vein in all tepals whereas in bifur15 and bifur16 three to seven occurred in the female tepals and one to three in the male tepals.

However, the lack of a distinct boundary to the area of the decay cells in female plants grouped bifur15 and bifur17 together. This indicates that the distinction from *R. praeacutus* on the basis of a distinct boundary is not always valid in terms of the female plants. Male plants did all exhibit clear delineation of the decay cell area but wider sampling is indicated to test if this holds true for all populations. If the decay cell area is clearly delimited one can only be sure that the specimen is not *R. praeacutus*.

Thus this study indicates a constant group corresponding to *R. praeacutus* and a variable group that is not easily delimited. Bifur15 and bifur16 appear to correspond to *R. burchellii* which are said to have a wide altitudinal range (Linder, 1985) and bifur17 to *R. bifurcus* which is more closely related to *R. praeacutus* as indicated by positioning in the ordinations and cluster analyses. However, this study does not support specific status due to the lack of a unique combination of features in these two groups or the clear separation from other groups.

### **The *R. bolusii* group**

The early flowering group (Group E on the phenograms plus bolus21) could be differentiated from all but the *R. strobilifer* group in that a greater number of veins occurred in the bracts as evidenced from the striated appearance. The number of veins in the female bracts of *R. bolusii* was 17 to 23 and that in *R. strobilifer* 14 to 21 whereas the remaining groups displayed 4 to 14 veins. A greater number of veins were also present in the male bracts of these two groups with 12 to 13 being evident in *R. bolusii* and 10 to 17 in *R. strobilifer* with 3 to 9(-12) occurring in the remaining groups.

*R. bolusii* could be differentiated from *R. strobilifer* in other features of the bract. The characteristic bract of the former group is that of diagram d in fig 5, whereas diagram a is characteristic of the latter group. The decay cells are largely lost to either side of the mucro in *R. bolusii* whereas they remain attached in *R. strobilifer*. This loss of decay cells was not unique as some specimens of the middle group also displayed this feature.

A distinct delineation between the coriaceous section and decay cells occurs in *R. bolusii* whereas that in *R. strobilifer* is indistinct. The former group possesses a stout mucro whereas the latter is muticous. This agrees with previous studies in which characters used to separate these two species include stoutly mucronate bracts with a rounded apex in *R. bolusii* and muticous bracts with an obtuse apex in *R. strobilifer* (Pillans, 1928; Linder, 1985).

*R. bolusii* was also noted to possess more veins in tepals, with three to five but no single veins occurring. The only other items to display three or more veins occurred in the '*R. burchellii*' (bifur15 and bifur16) group as well as one item of the middle group (burch19). The latter was noted to often cluster with bolus21, bifur15 and bifur16 (figs. 11 and 13). The female anterior tepals of *R. strobilifer* all contained one vein only whereas in *R. bolusii* they all possessed five.

The anterior tepal of male flowers of *R. bolusii* were found to be glabrous which agrees with Pillans' (1928) description, but the females showed apical villousness in certain cases and were glabrous in others.

*R. strobilifer* and *R. bolusii* have previously been separated using the feature of perianth length (Pillans, 1928; Linder, 1985) with *R. bolusii* having a perianth of 6-8 mm long and *R. strobilifer* of 5 mm long but this character was not shown to distinguish these groups in this study.

### Evolutionary interpretation of cladogram

An evolutionary hypothesis based on the cladogram (fig. 15) could be the origin of the species at low altitude with two groups evolving. One of these groups, the *R. bifurcus*/*R. burchellii* group became ancestral to the species that were able to colonise higher altitudes with the line corresponding to *R. bifurcus* (bifur17) giving rise to the *R. strobilifer* clade and the line corresponding to *R. burchellii* (bifur 15 and bifur16 on cladogram) being ancestral to the *R. bolusii* clade.

## **CONCLUSION**

This study indicates the possible existence of three groups, namely *R. bolusii*, *R. praeacutus* and *R. strobilifer* with *R. insignis* being part of the latter group. In addition, bifur15 and bifur16 appear to correspond to *R. burchellii* which is said to have a wide altitudinal range (Linder, 1985) and bifur17 to *R. bifurcus*. However, this study does not support specific status due to the lack of a unique combination of features in these two groups or the clear separation from other groups. A central group containing a mixture of states characteristic of the outerlying groups also prevents clear delimitation of all the groups.

Much greater sampling is now required so as to obtain a clearer picture of infraspecific variation and to test the constancy of the characters that appear to be indicating the various groups. In addition, seeds should be taken later in the season to test whether the difference in seed wall morphology consistently separates *R. bifurcus* from the remaining groups as indicated in a study by Linder (1984).

## **Acknowledgements**

I would like to thank Prof. H.P. Linder for supervision and assistance with this project.

## REFERENCES

- Abbot L.A., Bisby F.A. and Rogers D.J. (1985); Taxonomic Analysis in Biology: Computers, Models and Databases; Columbia University Press.
- Crisp M.D. and Weston P.H. (1993); Geographic and ontogenetic variation in morphology of Australian Waratahs (*Telopea*; Proteaceae); Syst. Bot. 42(1): 49-76.
- Cutler D.F. (1969); Anatomy of the Monocotyledons Vol IV: Juncales; Clarendon Press.
- Dahlgren R. and Clifford H.T. (1982); The Monocotyledons: A Comparative Study; Academic Press.
- Davis J.I. and Manos P.S. (1991); Isozyme variation and species delimitation in the *Puccinellia nuttalliana* complex (Poaceae): An application of the phylogenetic species concept; Syst. Bot. 16(3): 431-445.
- Davis J.I. and Nixon K.C. (1992); Populations, genetic variation, and the delimitation of phylogenetic species; Syst. Bot. 41(4): 421-435.
- DeQueiroz K and Donoghue M.J. (1988); Phylogenetic systematics and the species problem; Cladistics 4: 317-338.
- Duncan T and Baum B.R. (1981); Numerical phenetics: Its uses in botanical systematics; Ann. Rev. Ecol. Syst. 12: 387-404.
- Faith D.P. and Cranston P.S. (1991); Could a cladogram this short have arisen by chance alone?: on permutation test for cladistic structure; Cladistics 7: 1-28.
- Felsenstein J (1985); Confidence limits on phylogenies: an approach using the bootstrap; Evolution 39: 783-791.
- James F.C. and McCulloch C.E. (1990); Multivariate analysis in ecology and systematics: panacea or Pandora's box?; Ann. Rev. Ecol. Syst. 21: 129-166.
- Kunth C.S. (1841); Enumeratio Plantarum Vol III; Cotta; p. 398.
- Linder H.P. (1984); A phylogenetic classification of the genera of the African Restionaceae; Bothalia 15 (1&2): 11-76.
- Linder H.P. (1985); Conspectus of the African species of Restionaceae; Bothalia 15(3&4): 387-503.
- Masters M.T. (1865); Observations on the morphology and anatomy of the genus *Restio* Linn., together with an enumeration of the South African species; Jnl. of the Linnaean Society 8: 211-255

- Masters M.T. (1878); Restiaceae; In Monographic Phanerogamarium Vol I; A & C De Candolle (eds.); Masson; pp. 218-398.
- Masters M.T. (1897); Restiaceae; In Flora Capensis Vol VII; W. Thiselton-Dyer (ed); Lovell Reeve and Co. Ltd.
- Nixon K.C. and Wheeler Q.D. (1990); An amplification of the phylogenetic species concept; Cladistics 6: 211-223.
- Pillans N.S. (1928); The African genera and species of Restionaceae; Transactions of the Royal Society of South Africa 7: 207-440.
- Pillans N.S. (1942); New Species of South African Restionaceae; Transactions of the Royal Society of South Africa 29: 339-356.
- Pillans N.S. (1945); New and hitherto imperfectly known species of African Restionaceae; Transactions of the Royal Society of South Africa 30(3): 245-266.
- Pillans N.S. (1950); Restionaceae; Flora of the Cape Peninsula; R.S. Adamson and T.M. Salter (eds.); Juta and Co. Ltd.
- Rolfe F.J. (1993); NTSYS-pc Numerical Taxonomy and Multivariate Analysis System; Version 1.80.
- Sneath P.H.A. and Sokal R.R. (1973); Numerical Taxonomy; W.H. Freeman.
- Sanderson M.J. (1989); Confidence limits on phylogenies: the bootstrap revisited; Cladistics 5: 113-119.

## **APPENDIX I**

### **COMPLETE SET OF CHARACTERS**

1. Average diameter of the female stem
2. Minimum diameter of the female stem
3. Maximum diameter of the female stem
4. Average diameter of the male stem
5. Minimum diameter of the male stem
6. Maximum diameter of the male stem
7. Female branching pattern
  1. single only
  2. single to few branches
  3. highly branched with no singles
8. Male branching pattern
 

as for 7
9. Average female ultimate sheath length
10. Minimum female ultimate sheath length
11. Maximum female ultimate sheath length
12. Average female ultimate sheath length including mucro
13. Minimum female ultimate sheath length including mucro
14. Maximum female ultimate sheath length including mucro
15. Average female penultimate sheath length
16. Minimum female penultimate sheath length
17. Maximum female penultimate sheath length
18. Average female penultimate sheath length including mucro
19. Minimum female penultimate sheath length including mucro
20. Maximum female penultimate sheath length including mucro
21. Average male ultimate sheath length
22. Minimum male ultimate sheath length
23. Maximum male ultimate sheath length
24. Average male ultimate sheath length including mucro
25. Minimum male ultimate sheath length including mucro
26. Maximum male ultimate sheath length including mucro
27. Average male penultimate sheath length
28. Minimum male penultimate sheath length
29. Maximum male penultimate sheath length
30. Average male penultimate sheath length including mucro
31. Minimum male penultimate sheath length including mucro
32. Maximum male penultimate sheath length including mucro
33. Sheath mucro
  1. cylindrical spike that extends onto the main body of the sheath
  2. cylindrical spike with a tapered base
  3. cylindrical spike that flattens basally becoming membranous and held together only by veins
  4. flat membranous spike held together by veins

#### **FEMALE SPIKELET**

34. Number of spikelets per culm
35. Minimum number of spikelets per culm
36. Maximum number of spikelets per culm

37. Average total length of spikelets (as measured from base of spathe)
38. Minimum total length of spikelets
39. Maximum total length of spikelets
40. Average spikelet length (as measured from the base of the bracts)
41. Minimum spikelet length
42. Maximum spikelet length
43. Average width as measured halfway along spikelet
44. Minimum halfway width
45. Maximum halfway width
46. Average basal quarter width
47. Minimum basal quarter width
48. Maximum basal quarter width
49. Average apical quarter width
50. Minimum apical quarter width
51. Maximum apical quarter width
52. Average spathe length
53. Minimum spathe length
54. Maximum spathe length
55. Average spathe length including mucro
56. Minimum spathe length including mucro
57. Maximum spathe length including mucro
58. Spikelet apex
  1. rounded
  2. elliptic
  3. obtusely angled
  4. acutely angled
59. Spikelet base
 

as for 58
60. Position of widest diameter on spikelet
  1. basal
  2. midway
  3. apical
61. Number of bract rows
62. Bract length
63. Basal bract width
64. Widest bract width
65. Bract apex at distal edge of decay cells
  1. rounded
  2. elliptic
  3. widely obtuse
  4. narrowly obtuse - 90° - acute
66. Bract apex at distal edge of coriaceous section
 

as for 65



67. Bract mucro
0. muticous
  1. slight ridge
  2. forms hook that arises within decay cell area
  3. forms slender point with base that tapers onto coriaceous section
  4. forms hard broad based point with the base arising from the coriaceous section
68. Mucro length
69. Length of distal decay cell area
70. Boundary between distal decay cells and coriaceous section
0. unclear
  1. clear
71. Persistence of decay cells
1. very membranous and largely lost
  2. largely intact
72. Number of veins per bract
73. Number of flowers per spikelet
74. Flower length
75. Anterior tepal villousness in the apical region
0. none
  1. short tuft of very few villi that extend from the tip
  2. slight apical villousness
  3. medium apical villousness
  4. dense apical villousness
76. Anterior tepal venation of outer whorl
0. no prominent veins
  1. central vein only
  2. 3 veins
  3. 5 veins
  4. 7 veins
77. Venation of interior whorl - lateral tepals
0. no large veins
  1. central vein only
  2. 3 veins
  3. 5 veins
78. Venation of interior whorl - posterior tepal  
as for 77

#### MALE SPIKELET

79. Number of spikelets per culm
80. Minimum number of spikelets per culm
81. Maximum number of spikelets per culm
82. Average total spikelet length as measured from base of spathe
83. Minimum total spikelet length
84. Maximum total spikelet length
85. Spikelet length as measured from the base of the bracts
86. Minimum spikelet length
87. Maximum spikelet length
88. Average halfway width of spikelet
89. Minimum halfway width of spikelet
90. Maximum halfway width of spikelet

91. Average basal quarter width
92. Minimum basal quarter width
93. Maximum quarter basal width
94. Average apical quarter width
95. Minimum apical quarter width
96. Maximum apical quarter width
97. Average spathe length
98. Minimum spathe length
99. Maximum spathe length
100. Spikelet apex  
as for 58
101. Spikelet base  
as for 58
102. Position of the widest diameter on spikelet  
as for 60
103. Number of bract rows
104. Bract length
105. Bract basal width
106. Maximum bract width
107. Bract apex at distal edge of decay cell area  
as for 65
108. Bract apex at distal edge of coriaceous section  
as for 65
109. Bract mucro  
as for 67
110. Length of mucro
111. Length distal decay cell area
112. Boundary between the distal decay cells and the coriaceous section  
as for 70
113. Persistence of decay cells  
as for 71
114. Number of veins per bract
115. Number of flowers per spikelet
116. Flower length
117. Anterior tepal villousness in the apical region  
as for 75
118. Anterior tepal venation of outer whorl  
as for 76

## **APPENDIX II**

### **SELECTED SET OF NON-OVERLAPPING CHARACTERS**

1. Average diameter of the female stem
2. Average diameter of the male stem
3. Female branching pattern
  1. single only
  2. single to few branches
  3. highly branched
4. Male branching pattern
  - as for 3
5. Average length of the female ultimate sheath
6. Average length of the male ultimate sheath
7. Sheath mucro
  1. cylindrical spike that extends onto the main body of the sheath
  2. cylindrical spike with a tapered base
  3. cylindrical spike that flattens basally becoming membranous and held together only by veins
  4. flat membranous spike held together only by veins

#### **FEMALE SPIKELET**

8. Number of spikelets per culm
9. Total length of spikelet as measured from the base of the spathe
10. Ratio of spikelet length as measured from the base of the first bract row to total spikelet length
11. Ratio of width as measured halfway along spikelet to total spikelet length
12. Ratio of width as measured a quarter of the way from the apex to total spikelet length
13. Ratio of the width as measured a quarter of the way from the base to total spikelet length
14. Ratio of the spathe length to total spikelet length
15. Apex of spikelet
  1. rounded
  2. elliptic
  3. obtusely angled
  - 4.1. widely acute
  - 4.2. narrowly acute
16. Base of spikelet
  - as for 15
17. Position of the widest diameter
  1. basal
  2. midway
  3. apical
18. Number of bract rows
19. Bract length
20. Ratio of the basal bract width to the length of the bract
21. Ratio of the widest part of the bract to the length

22. Bract apex at the distal edge of the decay cells
  1. rounded
  2. elliptic
  3. very widely obtuse
  4. narrowly obtuse - 90° - acutely angled
23. Bract apex at the distal edge of the coriaceous section  
as for 22
24. Bract mucro
  0. muticous
  1. slight ridge
  2. hook that arises within the decay cell area
  3. slender point with base tapering onto coriaceous section
  4. hard broad based point with base arising from the coriaceous section
25. Ratio of mucro length to bract length
26. Ratio of length of decay cell area to total bract length
27. Boundary between decay cells and coriaceous section
  0. indistinct
  1. distinct
28. Persistence of decay cells
  1. very membranous and largely lost
  2. largely intact
29. Number veins per bract
30. Number of flowers per spikelet
31. Length of flowers
32. Villousness of the anterior tepal
  0. no villi
  1. short tuft of very few villi (1-3) that EXTEND from the tip of the tepal
  2. slight apical villousness
  3. medium apical villousness
  4. dense apical villousness
33. Anterior tepal venation
  0. no prominent veins
  1. central vein only
  2. 3 veins
  3. 5 veins
  4. 7 veins
34. Venation of lateral tepals of interior whorl  
as for 33
35. Venation of posterior tepal of interior whorl  
as for 33

#### MALE SPIKELET

36. Number of spikelets per culm
37. Total length of spikelet as measured from the base of the spathe
38. Ratio of spikelet length as measured from the base of the bracts to the total cone length
39. Ratio of the width of the spikelet as measured halfway to the total length of the spikelet
40. Ratio of the width of the spikelet as measured a quarter of the way from the base to the total spikelet length

41. Ratio of the width of the spikelet as measured a quarter of the way from the apex to the total spikelet length
42. Ratio of the spathe length to the total length of the spikelet
43. Apex of spikelet  
as for 15
44. Base of spikelet  
as for 15
45. Position of widest diameter  
as for 17
46. Number of bract rows
47. Bract length
48. Ratio of basal bract width to bract length
49. Ratio of widest part of bract to bract length
50. Bract apex at distal edge of decay cells  
as for 22
51. Bract apex at distal edge of coriaceous section  
as for 22
52. Bract mucro  
as for 24
53. Ratio of mucro length to bract length
54. Ratio of length of decay cell area to bract length
55. Boundary between decay cells and coriaceous section  
as for 27
56. Persistence of decay cells  
as for 28
57. Number of veins per bract
58. Number of flowers per spikelet
59. Flower length
60. Villousness of anterior tepal  
as for 32
61. Venation of anterior tepal  
as for 33

### **APPENDIX III**

#### **CHARACTERS USED FOR CLADISTIC ANALYSIS**

1. Female branching pattern
  0. single only
  1. single to few
  2. many; no single culms
2. Male branching pattern
  0. single only
  1. single to few
  2. many; no single culms
3. Sheath mucro
  0. cylindrical spike extending onto main body of bract
  1. cylindrical spike with tapered base
  2. cylindrical spike with membranous base
  3. flat membranous spike

#### **FEMALE SPIKELET**

4. Number of spikelets per culm
  0. mostly one to two
  1. many
5. Total length of female spikelet
  0. less than 19mm
  1. greater than 19mm
6. Apex of female spikelet
  0. widely acute
  1. narrowly acute
7. Base of female spikelet
  0. rounded
  1. obtusely angled
  2. acutely angled
8. Position of widest diameter
  0. basal
  1. midway
  2. apical
9. Bract apex at distal edge of decay cells
  0. widely obtuse
  1. narrowly obtuse to acute
  2. elliptic
10. Bract apex at distal edge of coriaceous section
  0. rounded
  1. elliptic
  2. widely obtuse
  3. narrowly obtuse to acute
11. Bract mucro
  0. muticous
  1. slight ridge
  2. hook arising within area of decay cells
  3. slender spike with base on coriaceous section
  4. broad spike with base on coriaceous section

12. Boundary of decay cells
  0. indistinct
  1. distinct
13. Persistence of decay cells
  0. mostly lost
  1. mostly intact
14. Number of veins per bract
  0. less than six
  1. between six and twelve
  2. fourteen or more
15. Number of flowers per spikelet
  0. less than ten
  1. between ten and thirty
  2. more than forty
16. Villousness of anterior tepal
  0. non-villous
  1. very few short villi extending from tip of tepal
  2. slight apical villousness
  3. medium apical villousness
  4. dense apical villousness
17. Anterior tepal venation
  0. central vein only
  1. three veins
  2. five veins
  3. seven veins
18. Venation of lateral tepal of interior whorl
  0. central vein only
  1. three veins
  2. five veins
19. Venation of posterior tepal of interior whorl
  0. central vein only
  1. three veins

#### MALE SPIKELETS

20. Number of spikelets per culm
  0. few
  1. many
21. Apex of spikelet
  0. rounded
  1. elliptic
  2. obtusely angled
  3. widely acute
  4. narrowly acute
22. Base of spikelet
  0. rounded
  1. obtuse
  2. acute
23. Position of widest diameter
  0. basal
  1. midway
  2. apical

- 24. Bract apex at distal edge of decay cells
  - 0. rounded
  - 1. elliptic
  - 2. widely obtuse
  - 3. narrowly obtuse to acute
- 25. Bract apex at distal edge of the coriaceous section
  - 0. rounded
  - 1. obtuse
  - 2. acute
- 26. Bract mucro
  - 0. mucous
  - 1. hook arising within area of decay cells
  - 2. slender spike with base at coriaceous section
  - 3. broad spike with base on coriaceous section
- 27. Boundary of decay cells
  - 0. indistinct
  - 1. distinct
- 28. Persistence of decay cells
  - 0. mostly lost
  - 1. mostly intact
- 29. Number of veins per bract
  - 0. three to five
  - 1. seven to eleven
  - 2. twelve to fifteen
  - 3. more than fifteen
- 30. Number of flowers per spikelet
  - 0. less than 24
  - 1. between 30 and 35
  - 2. more than 50
- 31. Villousness of anterior tepal
  - 0. non-villous
  - 1. few short villi extending from tip
  - 2. apically villous
- 32. Venation of anterior tepal
  - 0. no prominent veins
  - 1. central vein only
  - 2. three veins
  - 3. five veins



# APPENDIX IV

OTU	CHARACTER	1	2	3	4	5	6	7	8	9	10	11	12	13
1	0.86	1.00	2.00	2.00	2.00	5.00	6.00	2.00	1.40	25.58	0.81	0.22	0.14	0.18
2	0.90	0.62	2.00	2.00	5.58	6.00	6.00	4.00	1.25	23.05	0.84	0.27	0.20	0.18
3	1.20	1.00	2.00	2.00	7.30	6.50	6.50	2.00	1.60	22.10	0.79	0.26	0.13	0.22
4	1.20	1.30	2.00	2.00	8.00	9.00	9.00	4.00	2.00	26.10	0.85	0.26	0.14	0.18
5	1.00	0.78	2.00	2.00	6.00	7.10	7.10	4.00	1.00	19.10	0.94	0.39	0.26	0.24
6	1.20	1.00	1.00	2.00	6.60	7.20	7.20	4.00	2.20	24.80	0.75	0.27	0.13	0.19
7	1.00	0.90	2.00	2.00	8.40	7.80	7.80	2.00	4.00	14.50	0.88	0.35	0.22	0.30
8	1.40	999.00	1.00	999.00	8.50	999.00	999.00	4.00	1.00	25.10	0.81	0.43	0.22	0.29
9	999.00	1.30	999.00	1.00	999.00	10.00	10.00	4.00	999.00	999.00	999.00	999.00	999.00	999.00
10	1.30	999.00	2.00	999.00	10.10	999.00	999.00	2.00	1.40	20.60	0.93	0.37	0.19	0.35
11	1.80	999.00	2.00	999.00	999.00	999.00	999.00	2.00	1.90	21.20	0.74	0.20	0.13	0.19
12	1.50	1.10	2.00	3.00	999.00	999.00	999.00	2.00	1.20	18.60	0.83	0.33	0.17	0.30
13	1.20	1.10	2.00	2.00	7.80	6.30	6.30	4.00	1.50	21.40	0.81	0.26	0.14	0.25
14	1.00	0.95	2.00	2.00	6.70	6.70	6.70	4.00	2.30	17.10	0.82	0.32	0.16	0.28
15	1.20	1.50	2.00	2.00	9.20	9.75	9.75	1.00	6.30	15.40	0.90	0.25	0.13	0.22
16	1.10	1.50	2.00	2.00	999.00	999.00	999.00	1.00	6.80	11.90	0.91	0.27	0.18	0.19
17	1.40	1.30	2.00	2.00	10.00	11.00	11.00	4.00	9.60	13.60	0.88	0.31	0.18	0.26
18	0.80	0.70	2.00	1.00	4.70	2.80	2.80	4.00	2.80	10.70	0.85	0.30	0.18	0.25
19	1.00	0.90	2.00	2.00	7.80	7.10	7.10	4.00	3.30	14.90	0.89	0.24	0.14	0.20
20	0.95	999.00	2.00	999.00	7.30	999.00	999.00	4.00	3.40	14.00	0.88	0.28	0.14	0.26
21	1.40	1.30	1.00	1.00	9.00	9.40	9.40	4.00	6.00	15.50	0.79	0.30	0.17	0.23
22	1.00	1.00	2.00	2.00	8.80	7.50	7.50	1.00	8.30	11.00	0.84	0.34	0.18	0.25
23	1.00	999.00	2.00	999.00	8.30	999.00	999.00	2.00	6.40	15.60	0.81	0.22	0.12	0.19
24	1.10	0.70	1.00	2.00	11.90	7.90	7.90	1.00	6.90	16.56	0.85	0.33	0.14	0.26
25	0.70	999.00	3.00	999.00	6.30	999.00	999.00	1.00	1.60	12.40	0.89	0.27	0.10	0.24
26	0.64	999.00	3.00	999.00	5.00	999.00	999.00	1.00	1.30	14.80	0.84	0.24	0.14	0.18

999.00 = missing data

OTU	14	15	16	17	18	19	20	21	22	23	24	25	26
1	0.22	4.20	3.00	1.00	9.00	8.00	0.38	0.56	3.00	3.00	0.00	0.00	0.19
2	0.20	4.20	3.00	1.00	8.10	9.00	0.44	0.67	3.00	3.00	0.00	0.00	0.11
3	0.42	4.20	3.00	1.00	6.70	10.00	0.60	0.80	3.00	1.00	1.00	0.00	0.20
4	0.22	4.20	3.00	1.00	10.80	10.00	0.40	0.65	3.00	1.00	1.00	0.00	0.15
5	0.21	4.20	3.00	1.00	5.40	7.50	0.60	0.87	999.00	1.00	4.00	0.00	999.00
6	0.27	4.20	3.00	1.00	6.80	10.00	0.50	0.65	3.00	3.00	0.00	0.00	0.20
7	0.36	4.10	4.00	3.00	4.80	5.50	0.82	0.82	3.00	1.00	1.00	0.00	0.36
8	0.26	4.10	1.00	1.00	12.00	8.00	0.56	0.75	3.00	1.00	1.00	0.06	0.19
9	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00
10	0.25	4.10	4.00	3.00	4.70	7.00	0.79	0.86	999.00	1.00	4.00	0.13	999.00
11	0.43	4.20	4.00	3.00	4.00	8.00	0.63	0.81	999.00	1.00	4.00	0.19	999.00
12	0.39	4.10	4.00	2.00	5.00	6.00	0.83	0.83	999.00	1.00	4.00	0.22	999.00
13	0.29	4.10	4.00	2.00	4.30	10.00	0.40	0.60	999.00	2.00	4.00	0.23	999.00
14	0.30	4.20	4.00	2.00	4.70	9.00	0.39	0.61	999.00	1.00	4.00	0.16	999.00
15	0.32	4.20	4.00	3.00	3.40	6.50	0.31	0.69	2.00	4.00	4.00	0.18	0.11
16	0.53	4.20	4.00	3.00	3.30	6.00	0.50	0.67	2.00	2.00	4.00	0.12	0.12
17	0.29	4.20	4.00	3.00	4.40	7.00	0.50	0.64	4.00	4.00	2.00	0.14	0.14
18	0.34	4.20	4.00	2.00	4.00	5.50	0.55	0.55	2.00	2.00	0.00	0.00	0.15
19	0.42	4.20	4.00	3.00	3.40	7.00	0.50	0.57	3.00	1.00	2.00	0.14	0.14
20	0.35	4.10	4.00	2.00	4.50	6.00	0.42	0.75	999.00	1.00	1.00	999.00	999.00
21	0.54	4.20	4.00	2.00	3.70	7.00	0.71	0.71	3.00	1.00	2.00	0.06	0.20
22	0.24	4.10	4.00	2.00	4.00	6.50	0.38	0.54	4.00	4.00	3.00	0.22	0.09
23	0.21	4.10	4.00	2.00	4.20	6.50	0.38	0.54	4.00	4.00	3.00	0.18	999.00
24	0.28	4.10	3.00	1.00	4.50	6.00	0.50	0.58	4.00	4.00	3.00	0.20	0.15
25	0.30	4.20	3.00	1.00	4.30	4.50	0.67	0.78	4.00	4.00	3.00	0.22	0.11
26	0.37	4.20	3.00	1.00	3.40	8.00	0.25	0.44	4.00	4.00	3.00	0.19	0.13

OTU	27	28	29	30	31	32	33	34	35	36	37	38	39
1	0.00	2.00	20.00	16.00	5.50	0.00	1.00	1.00	1.00	3.00	16.15	0.76	0.34
2	0.00	2.00	18.00	23.00	6.00	0.00	1.00	1.00	1.00	2.25	17.10	0.74	0.32
3	0.00	2.00	21.00	11.00	7.00	0.00	1.00	2.00	2.00	2.80	14.20	0.68	0.37
4	1.00	2.00	15.00	26.00	6.50	2.00	1.00	1.00	1.00	3.00	22.00	0.72	0.37
5	1.00	1.00	11.00	5.00	8.00	2.00	1.00	2.00	2.00	3.50	15.20	0.78	0.26
6	0.00	2.00	14.00	12.00	6.50	0.00	1.00	1.00	1.00	4.80	13.50	0.79	0.32
7	0.00	2.00	7.00	6.00	6.00	3.00	2.00	2.00	1.00	3.80	10.20	0.79	0.34
8	0.00	2.00	7.00	43.00	7.50	0.00	1.00	1.00	1.00	999.00	999.00	999.00	999.00
9	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	2.00	21.00	0.73	0.42
10	1.00	1.00	20.00	7.00	6.00	0.00	3.00	3.00	3.00	999.00	999.00	999.00	999.00
11	1.00	1.00	20.00	11.00	7.50	3.00	3.00	2.00	2.00	999.00	999.00	999.00	999.00
12	1.00	1.00	23.00	8.00	7.00	3.00	3.00	3.00	3.00	2.50	10.40	0.86	0.54
13	1.00	1.00	14.00	5.00	7.50	3.00	2.00	1.00	2.00	2.00	14.00	0.79	0.41
14	1.00	1.00	9.00	5.00	7.50	3.00	1.00	1.00	1.00	2.80	14.00	0.73	0.32
15	0.00	2.00	9.00	1.00	9.00	0.00	2.00	3.00	3.00	18.50	13.30	0.87	0.27
16	1.00	2.00	12.00	1.00	9.00	0.00	4.00	2.00	3.00	12.00	13.80	0.80	0.29
17	0.00	2.00	7.00	5.00	6.00	4.00	1.00	1.00	1.00	16.00	13.40	0.75	0.34
18	1.00	2.00	6.00	4.00	5.00	0.00	1.00	1.00	1.00	1.20	9.70	0.87	0.44
19	1.00	2.00	14.00	2.00	6.50	0.00	3.00	2.00	2.00	4.30	14.20	0.75	0.32
20	1.00	1.00	12.00	5.00	5.50	2.00	1.00	1.00	1.00	999.00	999.00	999.00	999.00
21	1.00	2.00	17.00	3.00	6.50	4.00	3.00	3.00	3.00	9.60	15.00	0.71	0.32
22	0.00	2.00	4.50	7.00	5.00	0.00	1.00	1.00	1.00	9.00	11.10	0.90	0.31
23	0.00	2.00	4.00	5.00	5.50	1.00	1.00	1.00	1.00	999.00	999.00	999.00	999.00
24	0.00	2.00	5.00	10.00	5.00	1.00	1.00	1.00	1.00	10.60	8.80	0.85	0.35
25	0.00	2.00	5.00	6.00	5.00	1.00	1.00	1.00	1.00	999.00	999.00	999.00	999.00
26	0.00	2.00	5.00	4.00	6.00	1.00	1.00	1.00	1.00	999.00	999.00	999.00	999.00

OTU	40	41	42	43	44	45	46	47	48	49	50	51	52
1	0.08	0.30	0.34	4.10	3.00	1.00	7.00	3.50	0.86	1.00	1.00	1.00	0.00
2	0.11	0.24	0.32	4.10	3.00	1.00	9.00	4.50	0.67	1.00	3.00	1.00	0.00
3	0.13	0.35	0.46	4.10	3.00	1.00	7.00	4.00	0.75	1.00	3.00	1.00	1.00
4	0.10	0.33	0.34	4.10	1.00	1.00	8.80	5.00	0.70	0.80	1.00	1.00	0.00
5	0.12	0.25	0.33	4.10	4.00	3.00	6.20	4.50	0.44	0.67	999.00	1.00	4.00
6	0.17	0.24	0.27	4.10	4.00	2.00	5.70	5.00	0.60	0.80	3.00	1.00	0.00
7	0.18	0.30	0.38	4.10	4.00	3.00	5.60	3.50	0.57	0.86	3.00	1.00	1.00
8	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00
9	0.17	0.32	0.34	4.10	1.00	1.00	9.50	5.00	0.80	1.00	3.00	1.00	0.00
10	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00
11	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00
12	0.30	0.52	0.46	1.00	4.00	3.00	4.00	4.50	0.78	0.78	999.00	1.00	4.00
13	0.18	0.41	0.34	1.00	4.00	3.00	5.60	4.50	0.44	0.89	999.00	1.00	1.00
14	0.14	0.36	0.35	1.00	4.00	3.00	5.90	5.00	0.60	0.80	999.00	1.00	4.00
15	0.16	0.23	0.24	4.20	4.00	2.00	5.00	3.50	0.86	1.00	2.00	1.00	4.00
16	0.20	0.28	0.43	4.10	4.00	3.00	4.50	6.00	0.58	0.67	2.00	3.00	4.00
17	0.12	0.31	0.34	4.10	4.00	3.00	5.70	4.50	0.67	0.78	4.00	3.00	2.00
18	0.23	0.39	0.26	1.00	4.00	3.00	5.40	4.00	0.50	0.75	1.00	1.00	0.00
19	0.15	0.33	0.30	4.10	4.00	3.00	5.70	4.00	1.00	1.00	999.00	1.00	2.00
20	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00
21	0.13	0.34	0.38	2.00	4.00	3.00	5.40	5.00	0.80	0.80	1.00	1.00	4.00
22	0.15	0.26	0.29	4.10	4.00	2.00	7.00	5.00	0.30	0.50	4.00	3.00	3.00
23	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00
24	0.19	0.28	0.35	4.10	3.00	3.00	5.00	3.00	0.50	0.83	4.00	3.00	3.00
25	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00
26	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00	999.00

